

**RAPD DETECT NO SOMACLONAL VARIATION IN CRYOPRESERVED CULTURES  
OF *PINUS ROXBURGHII* SARG.**

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**REFERENCES**

- Alberto F., Santos R., Leitao J. M. (1997). DNA extraction and RAPD markers to assess the genetic similarity among *Gelidium sesquipedale* (Rhodophyta) populations. *Journal of Phycology*, 33: 706-710.
- Aronen T. S., Krajinakova J., Haggman H., Ryyänen L. A. (1999). Genetic fidelity of cryopreserved embryogenic cultures of open-pollinated *Abies cephalonica*. *Plant Science*, 142: 163-172.
- Bonga J. M., Pond S. E. (1991). Adventitious shoot formation in cultures of 30-year old *Larix decidua*, *L. leptolepis*, and *L. laricina* trees. *Plant Cell, Tissue and Organ Culture*, 26: 45-51.
- Bonga J. M., von Aderkas P. (1993). Rejuvenation of tissues from mature conifers and its implications for propagation *in vitro*. In: Ahuja M. R., Libby W. J. (Eds.). *Clonal forestry I, Genetics and Biotechnology*, Springer-Verlag, Berlin-Heidelberg: 182-199.
- Bonga J. M. (1996). Frozen storage stimulates the formation of embryo-like structures and elongating shoots in explants from mature *Larix decidua* and *L. x eurolepis*. *Plant Cell, Tissue and Organ Culture*, 51: 195-200.
- Bonga J. M. (2004). The effect of various culture media on the formation of embryo-like structures in cultures derived from explants taken from mature *Larix decidua*. *Plant Cell, Tissue and Organ Culture*, 77: 43-48.
- Deverno L. L. (1995). An evaluation of somaclonal variation during somatic embryogenesis. In: Jain S. M., Gupta P. K., Newton R. J. (Eds.). *Somatic embryogenesis in woody plants*, Kluwer Academic Publishers, Dordrecht: 361-377.
- Ford C. S., Jones N. B., van Staden J. (2000). Cryopreservation and plant regeneration from somatic embryos of *Pinus patula*. *Plant Cell Reports*, 19: 610-615.
- Fourre J. L., Berger P., Niquet L., Andre P. (1997). Somatic embryogenesis and somaclonal variation in Norway spruce: morphogenetic, cytogenetic and molecular approaches. *Theoretical and Applied Genetics*, 94: 159-169.
- Godwin I. D., Sangduen N., Kuananuvatchaidach R., Piperidis G., Adkins S. W. (1997). RAPD polymorphism among variant and phenotypically normal rice (*Oryza sativa* var. *indica*) somaclonal progenies. *Plant Cell Reports*, 16: 320-324.
- Gupta P. K., Durzan D. J. (1985). Shoot multiplication from mature trees of Douglas fir and Sugar pine. *Plant Cell Reports*, 4: 177-179.
- Gupta P. K., Durzan D. J., Finkle B. J. (1987). Somatic polyembryogenesis in embryogenic cell masses of *Picea abies* (Norway spruce) and *Pinus taeda* (Loblolly pine) after thawing from liquid nitrogen. *Canadian Journal of Forest Research*, 17: 1130-1134.
- Haggman H. M., Ryyänen L. A., Arnoen T. S., Krajinakova J. (1998). Cryopreservation of embryogenic cultures of Scots pine. *Plant Cell, Tissue and Organ Culture*, 54: 45-53.
- Hargreaves C., Smith D. R. (1992). Cryopreservation of *Pinus radiata* embryogenic tissue. *Proceedings of International Plant Propagators Society*, 42: 327-333.
- Henry R. J., Nato A., de Buyser J. (1998). Genetic fidelity of plants regenerated from somatic embryos of cereals. In: Jain S. M., Brar D. S., Ahloowalia B. S. (Eds.). *Somaclonal variation and induced mutations in crop improvement*, Kluwer Academic Publishers, Dordrecht, Boston, London: 65-80.
- Hills P. N., van Staden J. (2002). An improved DNA extraction procedure for plant tissues with a high phenolic content. *South African Journal of Botany*, 68: 549-550.

- Hirsh A. G., Williams R. J., Meryman H. T. (1985). A novel method of natural cryoprotection. *Plant Physiology*, 66: 40-45.
- Hossain M. D., Konisho K., Minami M., Nemoto K. (2003). Somaclonal variation of regenerated plants in chilly pepper (*Capsicum annuum*). *Euphytica*, 130: 233-239.
- Isabel N., Tremblay L., Michand M., Tremblay F. M., Bousquet J. (1993). RAPD as an aid to evaluate the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana*. *Theoretical and Applied Genetics*, 86: 81-87.
- Isabel N. R., Boivin C., Levasseur P. M., Charest J., Bousquet J., Tremblay F. M. (1996). Occurrence of somaclonal variation among somatic embryo-derived white spruces (*Picea glauca*, Pinaceae). *American Journal of Botany*, 83: 1121-1130.
- Javouhey M., Daguin F., Ltouze R. (2000). Somatic embryogenesis-an efficient tool for date palm (*Phoenix dactylifera*) industrial micropropagation, characterization and genetic stability of original offshoots and regenerated plantlets by RAPD markers. *Acta Horticulturae*, 530: 237-241.
- Kaeppler S. M., Kaeppler H. F., Rhee Y. (2000). Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology*, 43: 179-188.
- Kartha K. K., Fowke L. C., Leung N. L., Caswell K. L., Hakman I. (1988). Induction of somatic embryos and plantlets from cryopreserved cell cultures of white spruce (*Picea glauca*). *Journal of Plant Physiology*, 132: 529-539.
- Laine E., Bade P., David A. A. (1992). Recovery of plants from cryopreserved embryogenic cell suspensions of *Pinus caribaea*. *Plant Cell Reports*, 11: 295-298.
- Lu Z. X., Reighard G. L., Baird G. L., Abbott W. V., Rajapakse S. (1996). Identification of peach rootstock cultivars by RAPD markers. *Horticultural Science*, 37: 127-129.
- Malabadi R. B., van Staden J. (2003). Somatic embryos can be induced from shoot apical domes of mature *Pinus patula* trees. *South African Journal of Botany*, 69: 450-451.
- Malabadi R. B., van Staden J. (2005a). Somatic embryogenesis from vegetative shoot apices of mature trees of *Pinus patula*. *Tree Physiology*, 25: 11-16.
- Malabadi R. B., van Staden J. (2005b). Role of antioxidants and amino acids on somatic embryogenesis of *Pinus patula*. *In Vitro Cellular and Developmental Biology-Plant*, 41: 181-186.
- Malabadi R. B., van Staden J. (2005c). Storability and germination of sodium alginate encapsulated somatic embryos derived from the vegetative shoot apices of mature *Pinus patula* trees. *Plant Cell, Tissue and Organ Culture*, 82: 259-265.
- Malabadi R. B., van Staden J. (2005d). Breakthrough in Forest biotechnology. University of KwaZulu-Natal, Pietermaritzburg, South Africa news paper, March vol. 2 (3): 3.
- Malabadi R. B., Choudhury H., Tandon P. (2004). Initiation, maintenance and maturation of somatic embryos from thin apical dome sections in *Pinus kesiya* (Royle ex. Gord) promoted by partial desiccation and Gellan gum. *Scientia Horticulturae*, 102: 449-459.
- Malabadi R. B., Hills P. N., van Staden J. (2006). RAPD assessment of clonal identity of somatic seedlings derived from the vegetative shoot apices of mature *Pinus patula* trees. *South African Journal of Botany*, 72: 181-183.
- Malabadi R. B., Nataraja K. (2004). Cryopreservation and plant regeneration via somatic embryogenesis in *Clitoria ternatea* Linn. *Phytomorphology*, 54: 7-17.
- Malabadi R. B., Nataraja K. (2006). Cryopreservation and plant regeneration via somatic embryogenesis using shoot apical domes of mature *Pinus roxburghii* Sarg. trees. *In Vitro Cellular and Developmental Biology*, 42 (2): 152-159.
- Niino T., Sakai A., Yakuwa H., Nojiri K. (1992). Cryopreservation of *in vitro*-grown shoot tips of apple and pear by vitrification. *Plant Cell, Tissue and Organ Culture*, 28: 261-266.
- Park Y. G., Kwon G. S., Tay D. (2005). Cryopreservation for gene conservation of *Acer mono* Max. *Propagation of Ornamental Plants*, 5 (2): 78-83.
- Pierik R. L. M. (1991). Commercial aspects of micropropagation. In: Prakash J., Pierik R. L. M. (Eds.). *Horticulture - New Technologies and Applications*, Kluwer Academic Publishers, Dordrecht, The Netherlands: 141-153.
- Rani V., Raina S. N. (1998). Genetic analysis of enhanced axillary branching derived *Eucalyptus tereticornis* and *E. camaldulensis* plants. *Plant Cell Reports*, 17: 236-242.
- Rani V., Parida A., Raina S. N. (2001). Chromosome number dependent genome size and RAPD fingerprinting diagnostic for integrity of enhanced axillary branching-derived plants of ten forest tree species. *Acta Horticulturae*, 560: 531-534.
- Roth R., Ebert I., Schmidt J. (1997). Trisomy associated with loss of maturation capacity in a long-term embryogenic culture of *Abies alba*. *Theoretical and Applied Genetics*, 95: 353-358.

- Rout G. R., Das P., Goel S., Raina S. S. (1998). Determination of genetic stability of micropropagated plants of ginger showing random amplified polymorphic DNA (RAPD) markers. *Botanical Bulletin of Academia Sinica*, 39: 23-27.
- Shenoy V. B., Vasil I. K. (1992). Biochemical and molecular analysis of plants derived from embryogenic cultures of napier grass (*Pennisetum purpureum*). *Theoretical and Applied Genetics*, 83: 947-955.
- Smith D. R. (1997). The role of *in vitro* methods in pine plantation establishment: The lesson from New Zealand. *Plant Tissue Culture and Biotechnology*, 3: 63-73.
- Volk G. M., Walters C. (2006). Plant vitrification solution 2 lowers water content and alters freezing behavior in shoot tips during cryoprotection. *Cryobiology*, 52: 48-61.
- Westcott R. J. (1994). Production of embryogenic callus from nonembryonic explants of Norway spruce *Picea abies* (L.) Karst. *Plant Cell Reports*, 14: 47-49.
- Widholm J. M. (1972). The use of fluorescein diacetate and phenosafranin for determining viability of cultured plant cells. *Stain Technology*, 47: 189-194.
- Williams J. G. K., Kubelik A. R., Livak K. L., Rafalski J. A., Tingey S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18: 6531-6535.
- Zoriniants S. E., Nosov A. V., Monforte-Gonzalez M., Mendeszeel M., Loyola-Vargas V. M. (2003). Variation of nuclear DNA content during somatic embryogenesis and plant regeneration of *Coffea arabica* L. using cytophotometry. *Plant Science*, 164: 141-146.