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Source for the Production of Bioactive Compounds

Christopher Thome*

Department of Biology, Laurentian University, Ramsey Lake Road, Canada

Corresponding author: Christopher Thome, Department of Biology, Laurentian University, Ramsey Lake Road, Canada, E-mail: Thome_C@Zed.Cn

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Description

Tissue culture technology of cassava (*Manihot esculenta Crantz*) is a viable alternative to currently adopted techniques for mass propagation, germplasm conservation and genetic improvement. DNA fingerprints of axillary bud regenerated plants displayed monomorphic bands similar to mother plant, indicating homogeneity among the regenerated plants with donor mother plant. The effect of subculture frequency on genetic stability of axillary bud-derived regenerants and micropropagated plants was also assessed using SSR markers. All the SSR profiles from axillary bud regenerants and micropropagated plants were monomorphic and comparable to mother plants from subculture, confirming the genetic stability among clones and mother plants. At the 6th subculture, similarity indicators between the progenies and the mother plants ranged from 0.95 to 1.0 and such a similarity indicated a very low polymorphism. The dendrograms generated through Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis of the 6th subculture revealed 96% similarity amongst axillary bud regenerants and micropropagated plants with donor mother plant. This low polymorphism ratio between mother plants, axillary bud regenerants and micro-propagated plants indicates the little effect of somaclonal variations, the high genetic similarity between mother plants and progenies and demonstrates the reliability of this propagation system for cassava. These results suggest that direct organogenesis from the axillary buds is the safest method for regeneration of true-to-type plants and this system can be used for clonal mass propagation, germplasm conservation and genetic transformation of cassava.

Micro-Propagated Plants

Cassava (*Manihot esculenta Crantz*) is a staple food crop for more than a billion people in 105 sub-tropical and tropical countries. The tuberous storage roots are rich in carbohydrates and can be cooked or processed for human consumption. Several agronomic traits make cassava suitable as a subsistence crop for rural farmers. It is drought-tolerant, grows well on low-nutrient soils and requires minimal resource input to cultivate. In addition, the tubers can be left in the soil for up to 3 years after maturation without decay and harvested when needed, making it very useful food security crop. With the on-going climate change and looming global energy crisis, cassava is also an ideal crop for bioenergy generation, biomaterial production and animal feed due to its high biomass productivity and high starch quantity and quality. The global cassava harvest yield is about 277 million metric tonnes, of which Latin America, Asia and Africa account for 10%, 32% and 56%, respectively. This production is far much below the yield potential of cassava under near-optimum climatic conditions. Cassava is vegetatively propagated using field stem cuttings and low production is caused by unavailability or limited access to planting materials and infection by diseases transmitted through successive generations. To increase cassava production, biotechnology interventions are required to supply quality clean planting materials, development of high yielding and disease resistant varieties using both conventional breeding and transgenic technologies. Plant tissue culture is recognized as one of the key areas of biotechnology because of its potential use in mass propagation hence season-independent production of planting materials, *in vitro* conservation and as a tool for genetic improvement. For clonal propagation of elite materials, *in*

vitro conservation and Agrobacterium-mediated genetic transformation, a reliable and efficient *in vitro* system to regenerate phenotypically and genotypically identical plants is a pre-requisite. However, tissue culture environment and influence of culture conditions like plant growth regulators, culture media, type of explant, temperature, pH etc. induces somaclonal variation during the culture process ultimately leading to genomic changes in regenerated plants. Even at optimal levels, frequent transfers of cultures during micro-propagation can result in genetic variation, thus questioning the clonal fidelity of regenerated and micro-propagated plants. The occurrence of somaclonal variation is a potential drawback when the propagation of an elite variety is intended, where clonal fidelity is required to maintain the advantages of desired elite genotypes (*e.g*., superior growth, starch properties, disease resistance and other quality traits).

Genetic Integrity

In order to make this technology commercially viable, it is important to verify that plants obtained by micro-propagation are true-to-type to the parent plant from which they were derived. In comparison to various morphological, cytological and protein markers used in the detection of variation in tissue culture raised plants, molecular markers are more stable, heritable and highly reproducible. Several molecular markers such as RAPD, ISSR, SSR and RFLP-ISSR have been used to detect genetic uniformity and identify any potential soma-clonal variations in plants produced through micro-propagation. Of these, Simple Sequence Repeat (SSR) markers have advantages of high genomic abundance throughout the genome, co-dominant, locus-specific, greater reproducibility, high level of polymorphism, informative and strong discriminatory power. Many reports have documented the assessment of genetic fidelity of micro-propagated plants using microsatellite markers. However, there is no report on assessment of genetic integrity of *in vitro* regenerated cassava plants by SSR markers. Among the farmer-preferred cassava cultivars in East Africa, TME14 and Kibandameno are landraces ranked highest by farmers. They are being scored excellent for plant establishment, early maturation, high yield, mealiness, flavor, cooking qualities and market value. Additionally, TME14 is resistant to Cassava Mosaic Disease (CMD) and Kibandameno has a sweet pleasant flavor, thus making them ideal selection for growers. So far, *in vitro* regeneration of plants using axillary buds as explants and clonal fidelity of micro-propagated plants of cultivars TME14 and Kibandameno has not been done. The aim of this study therefore, was to assess the genetic fidelity of cassava plants regenerated from axillary buds using SSR markers. In addition, this study evaluated the effect of subculture frequency on genetic fidelity of axillary derived and micro-propagated cassava plants to clarify the variation pattern and frequency of somatic variation in the course of the subculture.

The effect of subculture frequency on genetic variations of axillary bud-regenerated plants was analyzed using 10 SSR markers. The number of bands varied from 2 to 4, with an average of 3 bands per SSR primer. These 10 SSR primers generated a total of 162 amplicons from all the six subcultures of axillary bud-derived plants and the band sizes ranged from 130-850 bp. The banding pattern of PCR amplified products from plants of subculture was monomorphic. The genetic similarities of the mother plant and sub-cultured plants based on SSR markers varied from 0.955 (mother plant and subcultured plants of 6th generation) to 1 (subcultured plants of 1^{st}–5^{th} generations) with an average value of 0.9775. Dendrogram analysis based on the Jaccard's similarity coefficient revealed 100% genetic similarity among the mother plants and its derivatives from 1st to 5th subcultures. At the 6th subculture, the mother plants and axillary bud regenerants were highly similar (similarity coefficient level was 1) for cultivars TME14 and Kibandameno. Only one variant was observed at the 6th subculture for cultivar TMS60444 at genetic similarity of 0.955 and the polymorphism level was 3.70%. This polymorphism was observed in one of the regenerants of cultivar TMS60444 using primer SRY78.