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## Inter Simple Sequence Repeat (ISSR) Markers as Reproducible Tools for Genetic Diversity Analysis of an Agroforestry Tree Species *Guazuma crinita* from Peruvian Amazon

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### Abstract

Agroforestry system is an efficient method for sustainable use and conservation of tropical trees. *Guazuma crinita* is an important fast-growing timber tree species for agroforestry systems in the Peruvian Amazon and little is known about its genetic variation. A molecular approach using inter-simple sequence repeat (ISSR) markers was applied to 45 genotypes of *G. crinita* from a collection of clones from a small and representative watershed in Peruvian Amazon. To obtain clear and reproducible bands, 30 ISSR primers and 2 parameters (annealing temperature, DNA concentrations) were screened. The optimal conditions for ISSR system for each 20  $\mu$ l PCR reaction mixture was composed of 10  $\mu$ l of 2 $\times$  PPP Master Mix, 0.5  $\mu$ l of respective ISSR primer, 2  $\mu$ l of DNA (50 ng/ $\mu$ l), 0.2  $\mu$ l of BSA and 7.3  $\mu$ l PCR H<sub>2</sub>O. Of the total 30 ISSR markers, seven primers produced well reproducible bands with optimal annealing temperature ( $T_a$ ) varying from 47 to 52°C. A total of 47 were amplified of which 44 were polymorphic (93.62%). The range of DNA amplification varied from 260 to 1700 bp. The general genetic differentiation ( $G_{st}$ ) was estimated to be 0.40 and the gen flow ( $Nm$ ) 0.75 alleles per generation. The analysis of molecular variation (AMOVA) assigned 100% of genetic variability to within provenances diversity. The UPGMA analysis did not distinguish provenances relationship suggesting a common gene pool. The genetic assessment suggests the collection of plant material for the establishment of genebank and in situ conservation strategies program for this tree species. ISSR markers were chosen because the technique is very simple, fast and it also requires small quantity of sample DNA.

**Keywords:** Genetic diversity, *Guazuma crinita*, inter-simple sequence repeat marker, sustainable conservation