

Genetic Relationships and Ploidy Levels of *Rubus* spp. of the Tropical Americas

Background

Several *Rubus* species and landraces are grown in Costa Rica and throughout the tropics. These possess important traits such as low acidity, high ORAC values, and dwarfism (Figs. 1 and 5). *R. adenotrichos* landraces are the most economically important, and are widely grown throughout the country. Little is known about the genetics of these plants, and because there is potential for improved cultivars, we studied the genetic relationships and ploidy for crossing genotypes. From the point of view of genetic resources, it is also important to study this material as these are highland blackberries that can be affected by global climate change.

Materials and Methods

R. adenotrichos, *R. urticifolius*, *R. miser*, and *R. glaucus* are tropical genotypes grown in Central and South America and were obtained from several local growers (Flores-Mora et al. 2005; Fig. 1). Three elite cultivars donated from the Corvallis Clonal Germplasm Repository were used as controls. 12 Genotypes representing 6 species were analyzed using 9 RAPD and 13 SSR markers. Nei's genetic distances were calculated and UPGMA was used to generate a dendrogram. Nuclei were isolated and analyzed by flow cytometry to determine ploidy (Meng and Finn 2002).

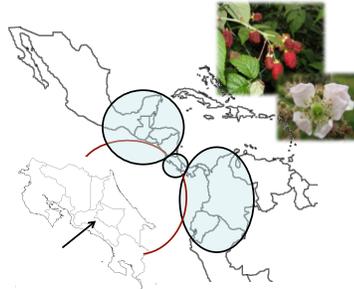


Figure 1. Map of regions where genotypes are found naturally (shown in blue), these include *R. adenotrichos*, *R. urticifolius*, *R. glaucus*, and *R. miser*. Map of Costa Rica expanded (red curves). Landraces used in this study were collected from growers in Zona de las Santos, San Jose Province (arrow).

Ramón Molina-Bravo¹
Irena Hilje-Rodriguez¹
Alfonso García-Piñeres²



¹Universidad Nacional de Costa Rica, Escuela de Ciencias Agrarias Apartado Postal 86-3000, Heredia, Costa Rica

²Universidad de Costa Rica, Centro de Investigación en Biología Celular y Molecular. Código Postal 11501-2060, San José, Costa Rica

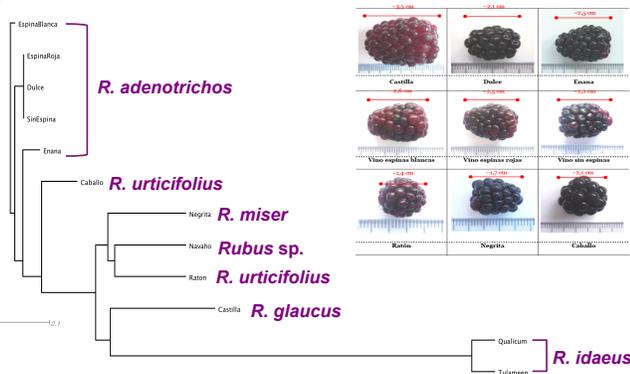


Figure 2. Genetic relationships among 12 *Rubus* genotypes. Dendrogram was generated by using 13 SSR markers, Nei's genetic distances, and the UPGMA procedure (left). Diversity in colors, shape and fruit sizes of tropical *Rubus* genotypes (upper right).

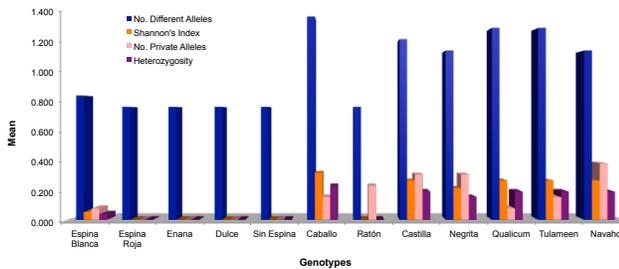


Figure 3. Allelic distribution, and marker diversity of 12 *Rubus* genotypes from 13 SSR markers.

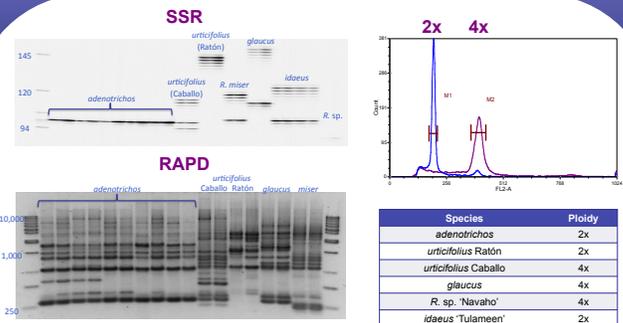


Figure 4. Genetic profiles of SSR and RAPD molecular markers of *Rubus* genotypes (left). Histograms of relative fluorescence intensities of *Rubus* nuclei that were detected as either 2x or 4x; 'Tulameen' and 'Navaho' cultivars used as 2x, and 4x controls, respectively (right).



Figure 5. Stems from prickly and non-prickly landraces of *R. adenotrichos* (left), *R. miser* flowers, the only genotype studied with lilac flowers (left), Enana is a dwarf landrace with short internodes, 5 to 8 cm, while other landraces range from 15 to 25 cm (right).

Results and Discussion

Both RAPD and SSR analyses showed very similar genetic distances and arrangements of the genotypes within the dendrogram. *R. glaucus* has been reported as a blackberry-raspberry hybrid (Jennings 1988). This is also supported by the marker analyses, as it is closest to the raspberry cultivars ('Tulameen', and 'Qualicum'; Fig. 2). Although Caballo and Ratón are the same species, Caballo was closer related to *R. adenotrichos* (Fig. 2). Additionally, histograms of fluorescent intensities show different ploidy between Caballo and Ratón (Fig. 4). Genetic profiles also suggested higher ploidy in Caballo because number of PCR products were higher in both SSRs and RAPDs (Figs. 3 and 4). Therefore taxonomic classification should be reexamined.

RAPD markers generated ≥ 100 bands per sample, while SSR markers generated up to 9 alleles per marker. Allelic diversity was low among *R. adenotrichos* genotypes, and highest within Caballo. For future crosses and study populations, *R. adenotrichos* landraces should be crossed with other genotypes to increase allelic diversity.

Acknowledgements

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References

Flores-Mora, D., Jiménez-Banillo, V., & Ortiz-Sanchez, F. (2005). Micropropagación, enraizamiento y aclimatación de mora (*Rubus* spp.) y su traslado al campo. In *Cultivo de la Mora, Innovaciones tecnológicas* (1st ed., pp. 65-75). Cartago: Editorial Tecnológica de Costa Rica.

Jennings, D. (1988). Raspberries and blackberries: their breeding, disease and growth. London: Academic.

Meng, R., & Finn, C. (2002). Determining ploidy level and nuclear DNA content in *Rubus* by flow cytometry. *Journal of the American Society for Horticultural Science*, 127(5), 767-775.