Transferring Simple Sequence Repeat Molecular Markers from a Model to Cultivated *Lotus* Species: Genetic Diversity in an Association Mapping Population of *Lotus tenuis*

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Transferring simple sequence repeat molecular markers from a model to cultivated *Lotus* species: genetic diversity in an association mapping population of *Lotus tenuis*

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Introduction

Lotus tenuis is a diploid (2n=12), out-crossing, self-incompatible, perennial species originating from the Mediterranean basin, North Africa, and Asia. On the other hand, *Lotus japonicus* has been selected as a model species because of its simple genetic conditions (diploid, self-crossing, and short ontogenetic cycle). *Lotus japonicus* is currently the focus of large multinational genome projects that periodically release outstanding products; these include sequencing of genomic DNA on a large scale, generating molecular markers, and constructing high-density linkage maps (Szczyglowski and Stougaard 2008). Furthermore, comparative genetic studies have demonstrated conservation of genome structure among model and crop species; this suggests that knowledge might be transferred effectively between these species. A population of 100 genotypes with divergent drought tolerance (tolerant and sensitive) was selected from INIA-Chile forage legume breeding program. This population has been physiologically and agronomically characterized under field and greenhouse drought conditions. It was recently genetically characterized with 88 simple sequence repeat (SSR) markers. Genetic and phenotypic information led to the identification of quantitative trait loci (QTLs) associated with drought tolerance of the species by association mapping analysis. The present study shows preliminary results of the molecular characterization performed with 88 SSRs developed in *L. japonicus* which amplified the *L. tenuis* genome. Genetic diversity analysis is presented in order to characterize the degree of information that SSRs provide and identify groups or structures within the population that can influence association mapping analysis.

Methods

Plant material

The association mapping population consisted of 100 genotypes from two *L. tenuis* populations naturalized in Chile that were previously selected for their divergent drought tolerance (Acuña *et al.* 2010). The drought-sensitive and drought-tolerant populations are referred to as Lt14 and Lt4, respectively. Study germplasm is made up of 50 individuals from each population.

Molecular characterization

The molecular characterization was carried out in the lab of Genomica Forestal at the Universidad de Concepción in Concepción, Chile. A shoot apical (undeveloped leaves) was extracted from each genotype and was deposited in a 2-mL Eppendorff tube and immediately frozen with liquid nitrogen. Genomic DNA was extracted with the DNeasy Plant Mini Kit (Qiagen). Eighty-eight SSR primers, previously developed in *L. japonicus*, were used for this assay (http://www.kazusa.or.jp/lotus/markerdb_index.html). Fifty-four primers were selected in previous species transferability studies of the *Lotus* genus developed in the LOTASSA Project (http://www.procisur.org.uy/ proyectos/pdfs/253692.pdf). The rest of the markers were selected from three studies which reported QTLs associated with agronomically important traits in *L. japonicus*. Annealing temperatures were adjusted with gradient PCR in a Veriti® Thermal Cycler. Polymerase chain reactions were performed using labeled microsatellite Tag under the following conditions: 1ng / µl of DNA, 10 x PCR Buffer, 50 mM $MgCl_2$, 10mM dNTPs, specific Primer Locus 10 ρmol/µl, Forward and Reverse Tag first 10 ρmol/µl, Taq DNA polymerase 5 U/µl, and 1 mg/ml BSA in a total volume reaction of 10 µl. Polymerase chain reaction was programmed for 5 min of initial melting at 95ºC followed by 35 cycles each at 94ºC for 30 s, annealing temperatures at 60ºC for 45 s, 72ºC for 20 s followed by 35 cycles each at 94ºC for 30 s, 50 ºC for 60 s, 72ºC for 60 s, and a final extension step at 60ºC for 30 min. The PCR products were genotyped with an ABI 3130 xl automated sequencer and scored with GeneMapper® V4.0 (Applied Biosystems Inc.).

Data analysis

The degree of information for each primer was determined by allele frequency, number of genotypes, number of alleles, and polymorphism information content (PIC). An analysis of molecular variance (AMOVA) was performed to estimate the contribution to the total variance caused by the populations from which the study population was derived (Lt4 and Lt14). Finally, a matrix of Nei genetic distances was estimated and a cluster analysis was performed. All the analyses were performed with the

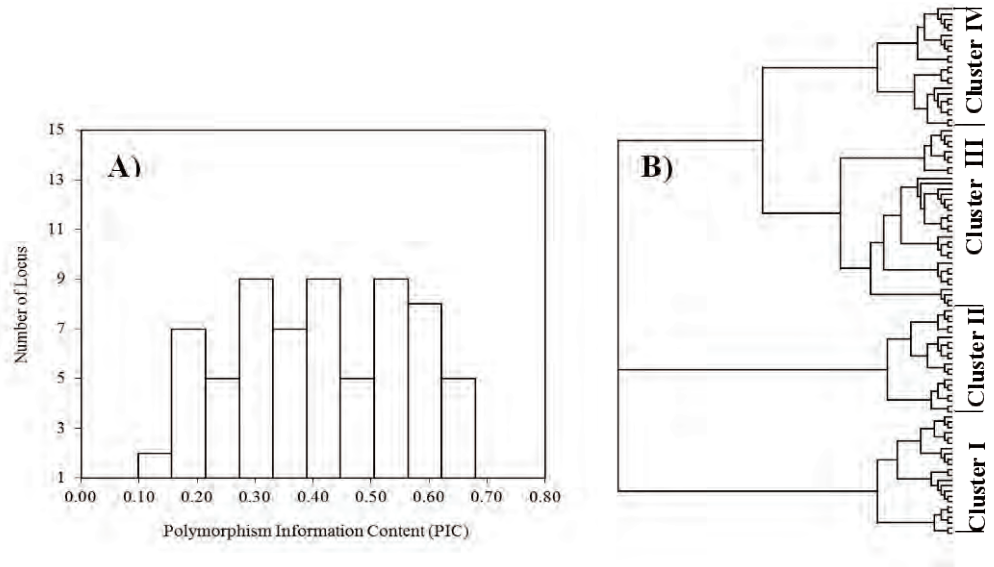

Figure 1. (A) Frequency distributions for polymorphism information content estimated in 75 SSR that amplifies the *Lotus tenuis* genome; (B) Dendrogram for the 100 *Lotus tenius* genotypes obtained from the unweighted neighbour joining analysis. Cluster I: 23 genotypes in total (48% from Lt-4 population); Cluster II: 20 genotypes in total (55% from Lt-4 population); Cluster III: 34 genotypes in total (58% from Lt-4 population) and Cluster IV: 23 genotypes in total (34% from Lt-4 population).

PowerMarker V3.25 software (http://statgen.ncsu.edu/ powermarker/).

Results

Two hundred and sixty-eight alleles were found by electropherogram analysis. No more than two alleles were considered per plant. The mean of alleles per locus was 3.6. Sixteen loci showed the lowest number of alleles (2) while TM1150 had the highest number (7). Polymorphism information content (Fig. 1A) is frequently used to evaluate the discriminatory power of a microsatellite locus. More than one equation defines PIC and the values range between 0 and 1. Higher PIC values indicate more information content. The mean PIC value for all the evaluated loci was 0.41. The highest PIC value was 0.68 (TM1150) and the lowest 0.10 (TM0014). By clustering individuals in their populations of origin (Lt14 vs. Lt4), AMOVA exhibited little genetic variance among them (2%). The greater part of the genetic variance (60%) was observed within each population of origin. The rest of the genetic variance (38%) was attributed to the individuals. Cluster analysis showed the formation of four clusters consisting of random individuals from the two populations of origin (Fig. 1B). This first characterization of the population structure is relevant for further association mapping studies.

Conclusion

The SSRs developed in *L. japonicus* amplified the *L. tenuis* genome without problems and showed a high discrimination capacity in the genetic diversity analysis. The association mapping population was structured in four clusters because to the founder populations (Lt-4 and Lt-14) they share some alleles.

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References

Acuña H, Inostroza L, Sánchez M, Tapia G (2010) Drought-tolerant naturalized populations of *Lotus tenuis* for constrained environments. *Acta Agriculturae Scandinavica, Section B - Plant Soil Science* **60**, 174 – 181.

Szczyglowski K, Stougaard J (2008) Lotus genome: pod of gold for legume research. *TRENDS in Plant Science* **13**, 515-517.