Use of Cross-Species Amplification Markers for Pollen-Medicated Gene Flow Determination in *Trifolium Polymorphum* Poiret

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Use of cross-species amplification markers for pollen-mediated gene flow determination in *Trifolium polymorphum* Poiret

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Introduction The species *Trifolium polymorphum* Poiret is endemic to Uruguay and is widespread in native grasslands throughout the country. Preliminary observations suggested that the aerial flowers are chasmogamous (open at maturity for potential cross-pollination) while the basal flowers are cleistogamous. Several approaches have been practised to determine the reproductive system of forage legumes by the aid of co-dominant markers (Real *et al*., 2004; Dalla Rizza *et al*., 2004). The aim of this study is to explore cross-species amplification as a quick approach to obtain co-dominant markers to study the breeding system of *T. polymorphum*.

Materials and Methods Ten single patches in the field of approx. 1 m^2 were selected for this study. Inside each patch, 20 individual plants were physically mapped, and subsequently transplanted to pots in a naturally lit glasshouse. For cross-*T .polymorphum* amplification, 50 ng of genomic DNA extracted as reported by Dalla Rizza *et al*., (2004) were used, following the amplification procedure described by Kölliker *et al*. (2001). Preliminary 4 perfect, 3 imperfect and 2 compound SSR classified markers were employed for the analysis using silver stain to reveal the amplicons in 8% non denaturing polyacrylamide gel electrophoresis.

Results From the 9 microsatellites primer pairs screened, 6 showed cross amplification success in *T. polymorphum* comparable in size to the product reported in *T. repens*. This is a highly unexpected value considering the differences in geographical origin of the two species and suggesting a probably remote common origin. As reported by Kölliker *et al.* (2001), this value observed for *T. polymorphum* is higher than *Trifolium pratense* L. and only surpassed by *Trifolium ambiguum* M.B. and *Trifolium nigrescens* Viv. At least 8 alleles were observed with the perfect microsatellite TRSSRA01H11 between distanced patches, more than observed in *T. repens* (Figure 1). Considering that no more than 2 alleles at a single locus would be expected in a diploid species probably this SSR has more than one locus, as also reported in *T. repens.* Between patches, high level of polymorphism was found meanwhile inside the patches the variability found among plants was low (lower than 20% in all the cases).

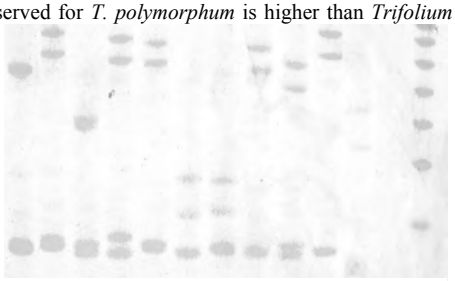

Figure 1 TRSSRA01H11 amplification on 10 *T. polymorphum* genotypes (first 10 lanes on left) and 1 genotype of *T. repens*

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Conclusions The high success rate of cross-species amplification obtained in *T. polymorphum* enables to extend the knowledge obtained in *T. repens* to *T. polymorphum* for plant breeding studies.

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