

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

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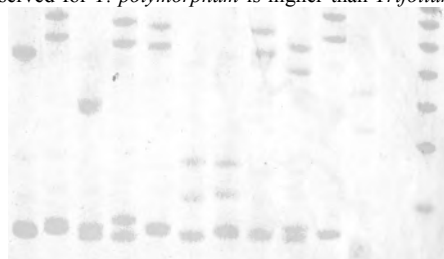
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**Introduction** The species *Trifolium polymorphum* Poiret is endemic to Uruguay and is widespread in native grasslands throughout the country. Preliminary observations suggested that the aerial flowers are chasmogamous (open at maturity for potential 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<sup>2</sup> 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.

### References

- Kölliker, R., E.S. Jones, M.C. Drayton, M.P. Dupal. & J.W. Forster (2001). Development and characterisation of simple sequence repeat (SSR) markers for white clover (*Trifolium repens* L.). *Theor Appl Genet* 102: 416-424.
- Real D., M. Dalla Rizza., K.H. Quesenberry & M. Echenique (2004) Reproductive and molecular evidence for allogamy in *Lotononis bainesii* Baker. *Crop Science*, Vol. 44 (2), 394-400.
- Dalla Rizza M., D. Real, K.H. Quesenberry & E. Albertini (2004) Plant reproductive system determination under field conditions based on codominant markers. (in press: *Journal of Genetics and Breeding*).