

Interspecific hybridisation between *Trifolium repens* L. and *T. isthmocarpum* Brot.

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Interspecific hybridisation in the genus *Trifolium* has proved to be difficult due to low seed yields, requirement for embryo rescue, lack of introgression, and variable hybrid fertility. Our team at AgResearch have created a wide range of interspecific hybrids using *T. repens* as the target species by manipulating the pre- and post-hybridisation barriers. A recent interspecific hybridisation involves *T. repens* and *T. isthmocarpum*. This diploid ($2n=2x=16$), annual wild relative of white clover has rarely been used in interspecific crosses. Hybridisation between the two species has been attempted in the past with limited success and only a few sterile triploid F1 hybrids were produced.

We have created fertile tetraploid F1 hybrids between *T. repens* and *T. isthmocarpum* without the use of embryo culture by manipulating the ploidy of *T. isthmocarpum*. Approximately 75 seedlings of $2x$ *T. isthmocarpum* from three different accessions were subjected to 0.1% colchicine for 12-14 hours and at least seven genotypes from each accession (a total of 33) were chromosome doubled.

Reciprocal hand pollinations were conducted between 17 chromosome doubled *T. isthmocarpum* ($2n=4x=32$) and six genotypes of *T. repens* from five cultivars. Crosses were successful where *T. repens* genotypes were used as female parents. Approximately 860 fully developed F1 seeds were obtained from an estimated 2500 pollinated florets. Thirty F1 seeds from various parental combinations were grown out. Somatic chromosome counts were made on a sample of 12 plants and all were tetraploid with three satellited chromosomes thus confirming hybridity of the plants. Meiotic chromosome configuration studies were conducted on a sample of eight plants. Meiotic configuration data at metaphase I revealed univalents, bivalents, trivalents and quadrivalents at various frequencies in different plants. The formation of multivalents in these F1 hybrids suggests homology between the chromosomes of these two species. Pollen staining of the F1 ranged from 78-97%. The high pollen stainability of these F1 hybrids may be due to the formation of balance gamete at anaphase I where only 16-16 chromosome disjunction was observed in approximately 30 cells. F1 hybrids are mostly like white clover in morphology with frequent nodal rooting and long stolons. F2 populations and BC1 progenies were developed efficiently allowing transfer of traits from *T. isthmocarpum* to white clover.