INVESTIGATION OF THE POSSIBILITY OF INTROGRESSION FROM *Trifolium ambiguum* M. Bieb. INTO *T. repens L.*

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ABSTRACT

The objective of this project was to investigate the possibility of introgression of stress resistance traits from *T. ambiguum* (A) into *T. repens* (R) by interspecific hybridisation, using two approaches. The first used *T. occidentale* Coombe (O) as a genetic bridge because this species has chromosome pairing homology to both the two other species. The second approach attempted direct integration of genomes from the two species through ploidy manipulation.

For the first approach, four crossing strategies used *T. occidentale* as a genetic bridge. Each started with different multispecies hybrids with various genomic contributions from the three parental species. The second approach began with 5x and 7x *T. ambiguum* x *T. repens* hybrids (ARRRR and AAARRRR). All the initial hybrids were repeatedly selfed, inter-crossed and backcrossed with colour-marked white clover and advanced progenies with reasonable levels of fertility were obtained in every strategy. Advanced hybrids were selected on the basis of flow cytometric ploidy estimation, phenotypes and somatic chromosome counts and were characterised for chromosome pairing and introgression events using both conventional and molecular cytogenetics. The advanced hybrid progenies were also grown in a sandpit to determine the relative expressions of the parental traits.

Chromosome analyses showed evidence of chromosome elimination, chromosome addition/substitution, allosyndetic pairing involving A-derived chromosomes and inter-specific genomic recombination. GISH analysis revealed that the genetic bridge strategy 1, which started with RRAO, gave a plant with four apparently large A-R and A-O chromosomal exchanges along with an A chromosome addition and a O-R substitution. No apparent signs of introgression were detected by GISH in the other strategies but introgression could not be ruled out because the sample was small and the morphology of the hybrids tested in the sandpit showed the expression of characters from both the parental species. These plants need to be characterised by using more genetic markers. The advanced progeny in the strategy based on direct integration of AxR genomes showed a low level of inter-specific chromosome pairing consistent with an absence of, or very low level of, introgression. The introgression revealed by GISH in the advanced progeny of RRAO hybrids provides evidence that using *T. occidentale* as genetic bridge

has worked by disrupting the genomic integrity in *T. repens*. The material having introgression has many applications from white clover improvement point of view. While direct hybrids with A & R genomes did not lead to any apparent introgression.

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ABBREVIATIONS AND TERMINOLOGY

The following abbreviations and terminology were used:

µl	microlitre
33	designation for 4x hybrid between *6x T. ambiguum* and 2x *T. occidentale* (AAAO).
434-1	designation for 3x hybrid between 4x *T. ambiguum* and 2x *T. occidentale* (AAO).
AA	acetic acid
AFLP	amplified fragment length polymorphism
AMV	alfalfa mosaic virus
BAR	Bridging *Ambiguum Repens.*
BAR09	the first progeny of original BAR hybrids bred and grown in 2009.
BAR10	the second progeny of original BAR hybrids bred and grown in 2010.
BC	backcross
BL	a 4x hybrid between between 4x *T. ambiguum* and 4x *T. occidentale* (AAOO).
BN	a 4x hybrid between between 4x *T. ambiguum* and 4x *T. occidentale* (AAOO).
CBC	congruity backcross
cm	centimetre
CYVV	clover yellow vein virus
DAPI	4',6-diamidino-2-phenylindole.
DNA	deoxyribonucleic acid
EBN	endosperm balance number
F_1	first filial generation
F_2	second filial generation
FC	flow cytometry

FISH	fluorescence *in situ* hybridization
GISH	genomic *in situ* hybridization
H-435	hybrid 435 with genomic composition, A^TA^TRR (4x)
Hybrid 70	designation for 4x hybrid, A^DA^TRR with two *T. ambiguum* genomes coming from diploid and tetraploid sources.
IIIs	trivalents
IIs	bivalents
Is	univalent chromosomal associations during anaphase-I
ITS	internal transcribed spacer
IVs	quadrivalents
MPV	mid parental value
mRNA	messenger RNA
MSAP	methylation sensitive amplification polymorphism
N	nitrogen
ng	Nanograms
NOR	nucleolar organizer region
OP	open-pollinated
Ph1	pairing homoeologous 1
PI	Propidium iodide
PMCs	pollen mother cells
PSV	peanut stunt virus
rDNA	ribosomal DNA
RET	*T. repens* with tetraploid genomic composition.
RNA	ribonucleic acid
RO	a white clover genotype named as Red One
ROS	designation for a 4x hybrid, AARR.
SC	self-compatible

SDR	second division restitution
SI	self-incompatible
SSC	saline sodium citrate
v/v	volume/volume
Vs	pentavalents
w/v	weight/volume
WCMV	white clover mosaic virus

Terminology for identifying different genomes/sub-genomes in different species

A	one sub-genome (x=8) from *T. ambiguum* (origin unspecified)
A^D	one sub-genome (x=8) from 2x *T. ambiguum*
A^T	one sub-genome (x=8) from 4x *T. ambiguum*
A^H	one sub-genome (x=8) from 6x *T. ambiguum*
O	one sub-genome (x=8) from 2x *T. occidentale*
R	one sub-genome (x=8) from white clover
R^P	*T. pallescens*-derived subgenome of *T. repens*
R^O	*T. occidentale*-derived subgenome of *T. repens*

Partial and mixed sub-genomes are designated as follows:

(A)	a partial sub-genome (x=unspecified number, 1-7) from *T. ambiguum*
(A_4)	a partial sub-genome (x=4) from *T. ambiguum*
(R/A)	a mixed sub-genome (x=~8) containing both white clover and *T. ambiguum* chromosomes.
(A/O)	a mixed sub-genome (x=~8) containing both *T. occidentale* and *T. ambiguum* chromosomes
(R_4/A_4)	a mixed sub-genome (x=8) containing four white clover and four *T. ambiguum* chromosomes.
(AO)	partial sub-genomes (x = 1-7) from *T. ambiguum* and *T. occidentale*.
(RAO)	partial sub-genomes (x = 1-7) from all the three species, white clover, *T. ambiguum* and *T. occidentale.*

A^O	a recombinant chromosome having *T. ambiguum* centromere with arms introgressed from *T. occidentale*
O^A	a recombinant chromosome having *T. occidentale* centromere with arms introgressed from *T. ambiguum.*