EFFECT OF PLOIDY ON INTERSPECIFIC HYBRIDISATION BETWEEN *Trifolium repens* L. AND RELATED SPECIES

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SYED WAJID HUSSAIN

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ABSTRACT

Two interspecific *Trifolium* hybrids were available at the time this project was initiated. The first hybrid (3*x* H-6909-5) was a sterile triploid obtained from a *T. repens* x *T. nigrescens* cross to transfer clover cyst nematode (*Heterodera trifolii* Goffart) resistance from *T. nigrescens* to *T. repens*. The second was a tetraploid hybrid (4*x* H-435) and its octoploid derivative (8*x* H-435), developed between 4*x* *T. ambiguum* and *T. repens* to transfer genes conferring longevity and virus resistance from *T. ambiguum* to *T. repens*. This was the first partially fertile hybrid reported between the two species. Chromosome doubling had increased pollen stainability in the octoploid clones (8*x* H-435).

The objectives of this project were to generate backcross progenies from these two hybrids at various ploidy levels, and to endeavour to achieve successful flow of genes between the parental species.

The triploid H-6909-5 ($2n=3x=24$) was highly sterile and produced no seed from approximately 3,000 reciprocal crosses to both parental species. It was chromosome doubled by an *in vitro* colchicine method using 0.1% and 0.05% colchicine for 48-72 h, depending on treatment. Three chromosome doubled plants (approximately 10% of the surviving meristems) from treatments with 0.05% colchicine and 48 or 60 h duration of application were obtained. Chromosome doubling resulted in a marked increase in fertility, as pollen stainability was increased from 9.9% in 3*x* H-6909-5 to an average of 89.2% (range 87.7-90.9%) in 6*x* H-6909-5. Subsequent backcrosses of 6*x* H-6909-5 and interbreeding of backcross derivatives resulted in an array of fertile hybrids at 4*x*, 5*x* and 7*x* levels, and some aneuploids. The occurrence of 7*x* BC_1F_1 progeny from the *T. repens* x 6*x* H-6909-5 (4*x* x 6*x*) cross is the first evidence of functional female 2*n* gametes in *T. repens*. The failure or success of different backcrosses, BC_1F_1 x BC_1F_1 and BC_1F_1 x 6*x* F_1 crosses supported a 2 maternal:1 paternal endosperm balance number (EBN) hypothesis to explain seed set in wide crosses. The EBN system will be helpful for predicting the success of future crosses if endosperm failure is the cause of hybrid embryo abortion. Meiotic chromosome pairing in F_1 and BC_1F_1 progeny

indicated the presence of allosyndetic pairing, suggesting that genetic exchange between the two species is possible.

T. nigrescens appeared to be a useful source of clover cyst nematode resistance. In the initial screening a mean number of 23.4 (range 0-150) cysts per plant was recorded for *T. nigrescens* in comparison to a mean number of 149.7 cysts per plant for *T. repens*. Rescreening of sixteen surviving *T. nigrescens* and five *T. repens* genotypes confirmed the initial screening results. H-6909-5 (3*x* and 6*x*) was also screened for clover cyst nematode resistance, and their resistance equalled that of the resistant *T. nigrescens* genotype. H-6909-5 (3*x* and 6*x*) had significantly fewer cysts/g root dry weight than the susceptible *T. nigrescens* and *T. repens* genotypes.

The second array of backcross progenies was generated from crosses involving 8*x* H-435 and *T. repens* and *T. ambiguum*. 8*x* H-435 was only cross fertile with *T. repens* and resulted in 145 seeds from 1,578 reciprocal crosses. Eleven out of 19 initially grown BC_1F_1 plants were all hexaploid ($2n=6x=48$). Mean pollen stainability for nine out of the 19 BC_1F_1's was 32.9% (range 19.1-64.9%). Meiotic chromosome pairing in the 6*x* BC_1F_1 plants averaged 2.1 univalents, 18.1 bivalents, 1.1 trivalents and 1.6 quadrivalents. From meiotic configurations it was not possible to conclude whether chromosomes of the two species had paired autosyndetically or allosyndetically but the occurrence of a high frequency of multivalents (up to three trivalents and four quadrivalents) indicated both types of pairing.

Backcrosses of 6*x* BC_1F_1 plants to *T. repens* resulted in 134 BC_2F_1 seeds from 760 crosses. Three out of seven initially grown BC_2F_1 plants were pentaploids ($2n=5x=40$). Pollen stainability averaged 59.3% (range 44.4-70.1%) for four 5*x* BC_2F_1 plants. On the other hand 6*x* BC_1F_1 x 6*x* *T. ambiguum* crosses did not produce any seed and only two pentaploid plants were obtained from 6*x* BC_1F_1 x 4*x* *T. ambiguum* crosses. One of these had 17.6% pollen stainability while the other did not produce normal inflorescences.

The difficulty encountered in generating 6*x* backcross progeny with 6*x* *T. ambiguum* was overcome by the creation of a fertile "bridging population". However the "fertile bridge" did not eventuate until after two generations of crossing. The 6*x*

BC_1F_1 plants were intercrossed and produced 114 BC_1F_2 seeds from 663 crosses. Two of the six initially grown BC_1F_2 plants were studied for somatic chromosome counts and were found to be hexaploid ($2n$=6x=48). The average pollen stainability was 40.8% for all six BC_1F_2 plants. One of these 6x BC_1F_2 plants was cross compatible as a female with 6x *T. ambiguum* and resulted 17 seeds from 318 reciprocal crosses.

Most of the 6x BC_1F_1 plants combined the rhizomatous and stoloniferous growth habit of the parental species and two of the ten 6x BC_1F_1 showed significant improvement in stolon number, stolon length, shoot dry weight and nodulation over 8x H-435. However, 6x BC_1F_2 are likely to be superior to 6x BC_1F_1 progeny, as they have exhibited better expression of the combined stoloniferous and rhizomatous growth habit, improved fertility, frequent nodal rooting and heavier nodulation than the BC_1F_1 progeny. Consequently the 6x BC_1F_1 plants can either be used directly in the selection programme or as a "fertile bridge" between the two parental species.

This work has resulted in the development of two arrays of fertile backcross progenies by manipulation of chromosome numbers and the production of a range of hybrid plants combining agronomic characteristics of the parent species in varying genome balances and at a range of ploidy levels. It is therefore concluded that initial sterility of the primary interspecific hybrids need not be a barrier to successful inter-breeding.

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