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Trifolium occidentale: A Valuable Genetic Resource for White Clover Improvement

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Trifolium occidentale: a valuable genetic resource for white clover improvement

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Introduction

Trifolium occidentale Coombe is a stoloniferous, diploid (2n=2x=16) perennial clover indigenous to Portugal, Spain, France, and the British Isles. It grows in relatively dry coastal habitats, in sand dunes and on shallow pocket of soil (Coombe 1961; Coombe and Morisset 1967). As the species grows naturally in saline, dry habitats, it is potentially a source of drought tolerance genes that could be used for the improvement of white clover (*Trifolium repens* L.) cultivars. Although *T. occidentale* is reported to be one of the progenitors of white clover (Williams *et al.* 2012), the 2x forms of *T. occidentale* cross with difficulty with white clover, resulting in near-sterile triploid hybrids. The two species were first crossed by Chou and Gibson (1968) and subsequently by Gibson and Beinhart (1969), and Chen and Gibson (1974). The relative success of producing F_1 hybrids was increased by use of colchicine doubled (4x) *T. occidentale*. Based on these reports, our objectives were: (1) to artificially double the chromosomes of *T. occidentale* using colchicine; (2) to use tetraploid (4x) *T. occidentale* as the pollen parent in crosses with white clover to produce large numbers of F_1 hybrids; (3) to evaluate hybrids both cytologically and morphologically; and (4) to develop advanced backcross and intercross progeny for future breeding and selection using white clover as the recurrent parent.

Methods

Chromosome doubling and chromosome counts

Seeds from fifteen diverse accessions of *T. occidentale* were treated in 0.1% aqueous colchicine solution for 12 hours. Eight to 10 week-old treated seedlings were transplanted to individual pots and were grown in a glasshouse. Chromosome counts were made from root-tip squashes and pollen mother cells, using our standard lab technique (Hussain and Williams 1997). Dry pollen shape provided a quick determination of the ploidy levels of the treated materials (Hussain and Williams 1997).

Pollination

Interspecific crosses were conducted by hand on potted plants in an insect-free glasshouse using the technique reported earlier (Hussain and Williams (1997). Initial F_1 crosses between white clover and 4x *T. occidentale* were made using white clover as the female parent. The reciprocal crosses were avoided because of the self-compatibility of most 4x *T. occidentale* genotypes. Self pollination of F_1s and subsequent generations were carried out by gently rolling a minimum of 2-4 flower heads of each plant between the thumb and fingers daily for three days.

Drought experiment

Stolon cuttings from 16 genotypes (four each from the two parental species, F_1 and BC_1F_1) were grown at a series of soil moisture levels (36%, 21%, 17% and 13% w/w) in 33cm x 33cm x 27.5 cm plastic boxes with five replicates to compare the relative responses of each set to soil drying under competition for light and nutrients. Data on root and shoot dry weights (DW) were recorded after 26 weeks.

Results

Chromosome doubling

Approximately 700 seedlings were recovered after treating 1076 germinating seeds from fifteen diploid (2x) *T. occidentale* lines with colchicine. The toxic effects of colchicine treatment reduced survival to 40-81% per line. Visual observations were recorded on plant growth and leaflet size as these two measures provide the first indication of chromosome doubling. At flowering, each plant was checked for dry pollen shape and classified as showing either complete doubling or mixed sectors of doubled and non-doubled tissue. Sectors of plants showing tetrahedral dry pollen were propagated vegetatively. Based on dry pollen evaluation, the different lines and different genotypes within the same line showed varying responses to colchicine doubling. In all, approximately 3% to 48% of recovered plants per line showed effective colchicine doubling. Of the 135 plants that were isolated on the basis of dry pollen shape as putative 4x plants, 25 were evaluated cytologically and all were confirmed, although six were 2x and 4x in different root tips. Later, vegetative cuttings were taken from chromosome doubled sectors (identified from dry pollen shape) and 4x clones were propagated vegetatively. These results showed a close correlation between dry pollen shape and actual chromosome numbers in the root tips. No aneuploids were found among the 25 plants evaluated.

Interspecific cross

Approximately 1100 F_1 seeds were produced, using six genotypes of white clover and 37 genotypes of 4x *T. occidentale*. From a total of 66 F_1seeds set for germination, 43 F_1 plants were grown to maturity and were used as male parents in first backcrosses (BC_1F_1) to 6 genotypes from four elite white clover cultivars. These crosses produced approximately 2700 BC_1F_1 seeds. Thirty six of the 43 F_1 plants were selfed. Five did not produce any seed and the remaining 31 yielded reasonable numbers of F_2 progeny ranging from 3-42 seeds from 2-3 heads. Pollen stainability estimates ranged from 21-78% in 28 of the 43 F_1 plants. The remaining 15 F_1 plants were not tested for pollen stainability. Twenty three different F_1 genotypes were intercrossed in 16 different combinations to produce intercrossed F_2 seeds. Seed numbers varied from cross to cross, ranging from 3-48 per cross and averaging 20 seeds per 100 florets.

Cytological evaluation

Eleven F_1 and 8 BC_1F_1 plants were evaluated for ploidy levels and were all 4x. Meiotic studies of 25 F_1 and 11 BC_1F_1 plants at metaphase I showed high frequencies of multivalents, indicating close homology between the chromosomes of the two parental species. Anaphase I chromosome disjunctions of 16-16 were observed in most F_1 and BC_1F_1 plants, suggesting that stable tetraploid progeny can be obtained from further crosses.

Drought experiment

White clover shoot DW declined more than *T. occidentale* and its hybrids, falling to 16% in the driest soil relative to the wettest soil, whereas *T. occidentale* declined only to 46% in the driest relative to the wettest soil. The F_1 and BC_1 hybrids showed intermediate reductions to 27% and 31%, respectively. White clover root DWs were the largest across all soil moisture levels, with *T. occidentale* smallest, and the hybrids intermediate. In all species, root DW increased as soil moisture fell from 36% to 21% and then remained largely unchanged with further drying. As the soil dried, root DW of *T. occidentale* and its hybrids increased by ~100%, and relatively much more than white clover (<30%). The BC_1F_1 hybrids appeared to be transgressive, showing a greater relative root growth response than either parent species.

Conclusion

Chromosome doubled (4x) *T. occidentale* proved to be efficient in crosses with white clover, resulting in large numbers of 4x F_1 hybrids. Meiotic analyses of both F_1 and BC_1F_1 revealed close homology between white clover and *T. occidentale* genomes, suggesting that genetic exchange occurs. A remarkable result was that the unselected BC_1F_1 hybrids have expressed strong performances against four highly selected, elite white clover genotypes. This raises the probability that selection of BC_1F_1 hybrids for performance would be expected to markedly enhance their potential at low soil moistures.

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