

## Interspecific hybridisation between *Trifolium repens* and *T. occidentale* for the improvement of drought tolerance in white clover

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### Introduction

*Trifolium repens* (white clover) is one of the most important forage legumes in temperate region of the world. Phylogenetically it is an allotetraploid between *T. pallescens* and *T. occidentale* (Williams *et al.*, 2012). Stands of white clover, although a perennial, often decline significantly by drought stress (Van Den Bosch *et al.*, 1993).

*T. occidentale* is a diploid ( $2n=2x=16$ ) stoloniferous perennial clover that grows naturally in saline, dry habitats and as such may be a useful source of genes to improve the drought tolerance of white clover cultivars (Hussain and Williams, 2013). Although white clover has been successfully hybridised with various annual and perennial *Trifolium* species (Williams, 1987), the resulting F<sub>1</sub> hybrids have not been effectively utilised as useful genetic material for the improvement of white clover cultivars. The objective of our current research was to evaluate F<sub>1</sub> and BC<sub>1</sub> hybrids between *T. repen* and *T. occidentale* for drought tolerance.

### Materials and Methods

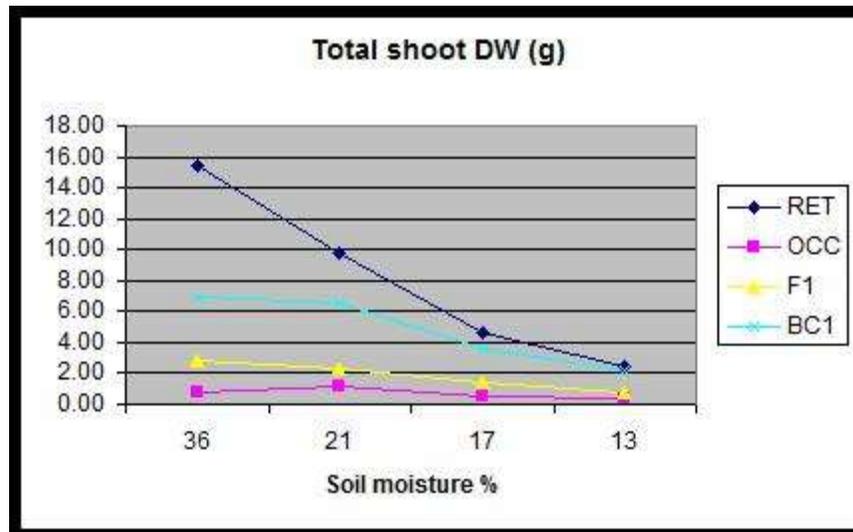
Stolon cuttings from 16 genotypes - four each from the parental species, F<sub>1</sub> and first backcrosses to white clover (BC<sub>1</sub>) - were grown at four soil moisture levels - 36% (field capacity), 21%, 17% and 13% - in plastic boxes to compare the relative responses of each set to soil drying under competition. The box internal dimensions were 33 cm x 33 cm x 27.5 cm (L x W x D). Total volume of the box was 29947.5 cc. Boxes were filled to 27 cm (29403 cc) with dry Egmont subsoil that has low organic matter, low fertility and a friable structure that was suitable for extracting intact roots at the end of the experiment. P and K were added to the top 7.5 cm layer of the soil with an amount of 8.3 g P and 3.5 g K per box. N was applied as ammonium nitrate at 50 kg/ha two times. This equated to 1.75 g ammonium nitrate per box. An amount of 0.5 g of MgSO<sub>4</sub> was applied with the first N application to supplement for Mg deficiency.

Each of the four moisture contents was represented by five replicate boxes. The boxes were arranged in a randomised complete block design. The lines were randomised in each box at 4 cm spacing (16 plants per box). Rooted cuttings were planted into the boxes. The cuttings were left to establish for one month before being transferred to a rain shelter. The boxes for the highest soil moisture treatment (approx. field capacity) were over-watered and allowed to drain for 24 hours. They were then weighed and were found to average 40.0 kg. These boxes were subsequently maintained at this weight by watering every 2-3 days throughout the duration of the experiment. The remaining boxes were allowed to progressively dry and, when wilting started, the boxes for the second treatment were weighed. These were watered 2-3 times daily to a weight of 32.5 kg. The third level remained un-watered until some irreversible wilting of leaves occurred (at 31.0 kg) and the fourth until significant leaf death occurred (30.0 kg). These weights were subsequently maintained by 2-3 daily watering. At the completion of the experiment these soil moisture contents were determined gravimetrically to be 36%, 21%, 17% and 13%, respectively. First and second harvests were carried out 5 and 10 weeks after the treatment and consisted leaf material only. Sixteen weeks after planting, a third and destructive harvest was carried out. Data were recorded on nine different morphological characteristics with emphasis on root (RDW) and shoot (SDW) dry weights. Data for each component were subjected to analysis of variance.

### Results and Discussion

**Shoot dry weights (SDW):** Results of cumulative top growth over three harvests are summarized in figure 1. White clover (RET) plants were the largest in all soil moisture treatments and *T. occidentale* (OCC) the smallest. The hybrids (F<sub>1</sub>) were intermediate, and the BC<sub>1</sub> hybrids progressively approached white clover in total growth as the soil dried, until they were almost identical in the driest soil treatment. The average relative SDW of white clover declined more than the other clovers, falling from 100 in the wettest to 16% in the driest soil. By contrast, *T. occidentale* declined much less, falling to 46% in the driest relative to the wettest soil. The *T. repens* x *T. occidentale* F<sub>1</sub> and BC<sub>1</sub> hybrids showed intermediate reductions to 27% and 31%, respectively. A simple ANOVA test has shown that the relative total SDW of *T.*

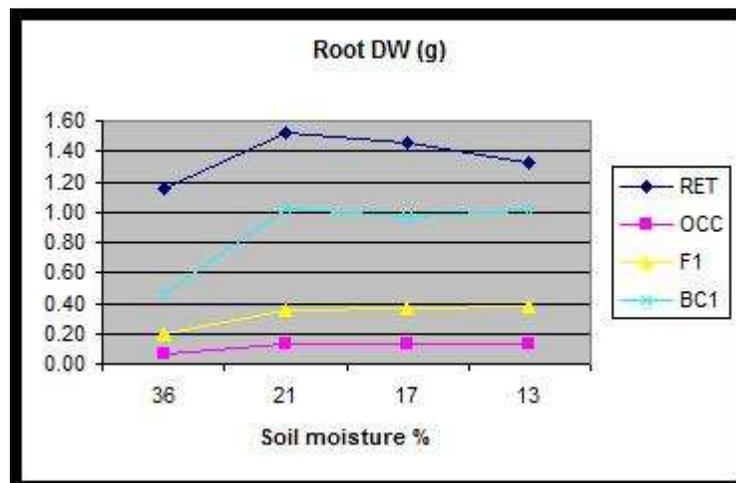
*occidentale* and the BC<sub>1</sub> hybrids in the driest treatment were significantly higher ( $P < 0.05$ ) than those of the white clovers. This is fully consistent with the conclusion that, relative to white clover, *T. occidentale* and its hybrids showed a smaller fall in shoot growth as the soil dried.



**Fig. 1:** Means of total shoot dry weights of white clover (RET), *T. occidentale* (OCC), F<sub>1</sub> and BC<sub>1</sub> at four different moisture levels.

**Root dry weights (RDW):** Actual root dry weights measured at the final harvest are presented in figure 2. White clover roots were the largest across all soil moisture levels, *T. occidentale* the smallest, and the hybrids were intermediate. In all species, RDW increased as soil moisture fell from 36% to 21% and then remained largely unchanged, except for a small reduction in white clover as moisture decreased further.

As with the other traits that are affected by propagule size, it may be more useful to compare relative responses than absolute responses. Root DWs relative to the moist treatment (36% soil moisture) are therefore also calculated. These show that *T. occidentale* and its hybrids responded relatively much more than white clover in root growth as the soil dried. The BC<sub>1</sub> hybrids appeared to be transgressive, having a greater relative response than either parent species.



**Fig. 2:** Means of total root dry weights of white clover (RET), *T. occidentale* (OCC), F<sub>1</sub> and BC<sub>1</sub> at four different moisture levels.

Root and shoot traits measured at the final harvest showed that BC<sub>1</sub> hybrids differed from white clover in the two driest treatments. In particular, petioles and stolons were longer and roots were thicker, leaf sizes were larger and numbers of growing points were higher in BC<sub>1</sub> hybrids at lower moisture levels. These results are indications that *T. occidentale* has imbued its hybrids with traits that may confer superior performance under competition at low soil moisture contents.

In almost all traits measured at the lowest soil moisture contents, the BC<sub>1</sub> hybrids apparently expressed transgressive genetic behaviour, i.e. they exceeded both parent species. Because the exact parental genotypes were not included as controls, this is indicative only. However, it is likely that when *T. occidentale* genomes are placed in white clover genetic

backgrounds, the expression of some very important and useful genes is enabled. These genes lead to the expression of traits that should provide superior performance in dry soils.

A remarkable result of this work is that the BC<sub>1</sub> hybrids have not been selected for anything and yet they have expressed strong relative performances against four highly selected, elite white clover genotypes. This raises the probability that selection of BC<sub>1</sub> hybrids for performance would be expected to markedly enhance their potential at low soil moistures.

At high soil moisture content, elite white clover out-grew *T. occidentale* and its hybrids. However, as soil moistures and competition for light were reduced, *T. occidentale* and its hybrids were relatively less reduced in total shoot growth and were increased more in root growth than white clover. In the driest treatments, the BC<sub>1</sub> hybrids, even without selection, were superior in most traits to either parent species. This is a strong indication that when genomes of *T. occidentale* are placed in an elite *T. repens* background, some very important genes are expressed that can confer superior performance in dry conditions.

## Conclusion

At high soil moisture content, elite white clover out-grew *T. occidentale* and its hybrids. However, as soil moistures and competition for light were reduced, *T. occidentale* and its hybrids were relatively less reduced in total shoot growth and were increased more in root growth than white clover. In the driest treatments, the BC<sub>1</sub> hybrids, even without selection, were superior in most traits to either parent species. This is a strong indication that when genomes of *T. occidentale* are placed in an elite *T. repens* background, some very important genes are expressed that can confer superior performance in dry conditions.

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