Drought Tolerance of Interspecific Hybrids between *Trifolium repens* and *Trifolium ambiguum*

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DROUGHT TOLERANCE OF INTERSPECIFIC HYBRIDS BETWEEN *Trifolium repens* AND *Trifolium ambiguum*

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Abstract

Hybrids between the stolonifeous white clover (*Trifolium repens* L., 2n=4x=32) and rhizomatous Caucasian clover (*T. ambiguum* M.Bieb, 2n=4x=32) have been produced. A backcross 2 (BC2) generation with white clover as the recurrent parent combines the growth habit of both parent species with the objective of increasing the persistency of large leaved *T. repens* varieties under grazing. *T. ambiguum* is more drought tolerant than *T. repens*. The drought tolerance of the hybrids in comparison with the parental species was compared in deep soil bins over a four week drought cycle. Soil moisture content, leaf relative water content (RWC), and leaf water potential were measured on plants subjected to drought and those watered normally and maintained at field capacity. *T. ambiguum* and the backcross hybrids were able to maintain a higher leaf RWC and leaf water potential than *T. repens* at comparable levels of soil moisture. The dry matter production of the hybrids and parental species was also compared in field plots sown with a perennial ryegrass companion. In the first harvest year, under a cutting regime, the yield of *T. repens* was highest and *T. ambiguum* lowest with the hybrids intermediate between the parents, with the BC2 approaching the yield of *T. repens*. These hybrids will be evaluated over further years and under grazing. The implications of these results for *T. repens* germplasm improvement programmes are discussed.

Keywords: *T. repens,* Caucasian clover**,** interspecific hybrids, drought tolerance, yield

Introduction

White clover *(Trifolium repens* L.*)* is the most important forage legume component of pastures in temperate northern Europe. It produces forage of high quality and in association with *Rhizobium trifolii* fixes atmospheric nitrogen, reducing the level of fertiliser nitrogen needed in mixed swards (Rhodes et al., 1999). Breeding programmes seek to improve traits such as seasonal and annual dry matter production, persistency and stress tolerance and while progress has been made in these areas, improvements are still necessary. *T. ambiguum* (Caucasian clover) is a rhizomatous but slow to establish perennial forage legume. Hybrids between the stoloniferous white clover (*T. repens* L) and rhizomatous *T. ambiguum* have been developed that combine the growth habits of both species (Abberton et al., 1998), with the objective of improving the persistency of large leaved white clover varieties under grazing. A backcross 2 generation has been produced that contains up to 7 % of its dry matter as rhizomes. *T.ambiguum* is also reported to be more drought tolerant than white clover. Improved drought tolerance of white clover is desirable to increase the locations over which it might be grown. This paper reports experiments designed to assess the drought tolerance of the hybrids in comparison with white clover and the growth of the hybrids in field plots under cutting.

Material and methods

The plant material was a *T. ambiguum* population (Ah1254) collected in Turkey in 1971 and *T. repens* variety Menna. Fertile F1 plants were used as the basis for two generations of backcrossing to *T. repens* as the recurrent parent (Meredith et al., 1995). Four backcross 1 and backcross 2 genotypes were chosen on the basis of possessing both stolons and rhizomes with the latter accounting for between 5-10% of the plants dry weight. This compares with nearly 60% in *T. ambiguum* (Abberton et al., 1998). These were cloned and used in the experiments described below.

Deep soil bins: Each bin of 1m x 1m x 1.4m was lined with a polyethylene bag with drainage holes in the bottom. The bins contained a 60cm layer of hardcore, 30cm coarse grit and an upper layer of 30cm JI potting compost. Each bin contained 4 clonal cuttings of each of the 4 genotypes. The bins were well watered and maintained at field capacity until the treatment period. A drought cycle was imposed over a 4 week period during which the bins were kept unwatered. Four bins comprising the four lines (*T. repens*, T. *ambiguum*, BC1 and BC2) were kept unwatered and four bins were watered once a week back to field capacity.

Field: Plots (2.7m x 1.5m) were sown on 1 September 98 with the intermediate perennial ryegrass variety Fennema at a rate of 25kg/ha. On 1 September 1998 each plot was planted with 8 clonal plants of each of 4 genotypes of either parent or the BC1 or BC2 generations. There were 4 replicate plots. Prior to sowing all plots received 100 kg/ha P_2O_5 and 200 kg/ha K_2O and 4 t/ha of lime. In spring 1999 plots received an additional 100 kg/ha P_2O_5 and 200 kg/ha K_2O and 40kg/ha N (34.5%N). Plots were lightly cut in 1998 and in 1999 were sampled five times at five-week intervals with a reciprocating blade mower (width of 0.23m) at a height of 3 cm. Herbage was weighed and a 100g sub-sample of herbage was taken from each plot for botanical analysis. This was separated into grass and legume component, dried in an oven at 80°C overnight, weighed and milled using a 1mm sieve.

Results

Over the 4 week drying cycle, the soil moisture content of the unwatered bins declined until they reached their lowest levels at weeks 3 and 4 (Figure 1). The leaf relative water content (RWC) of *T. repens* decreased (Figure 1) as the soil moisture content declined, and reached approximately 50% RWC by the end of the 4 week cycle. The leaf RWC of *T. ambiguum* plants was constant at more than 80% despite the soil moisture content decreasing to a similar level to *T. repens*. Leaf RWC of the BC1 and BC2 hybrids showed a response between those of *T. repens* and *T. ambiguum*. Leaf water potential of the plants in the watered bins remained greater than –10bar throughout the cycle. The leaf water potential in the unwatered bins decreased over time. Leaf water potential, after four weeks, was lowest in *T. repens* (-40bar), greatest in *T. ambiguum* (-8bar) and intermediate in the backcrosses (-20bar).

In the field experiment the DM yield of *T. repens* was greatest and *T. ambiguum* lowest with the hybrids intermediate (Table 1). The yield of the BC2 was greater than the BC1 but was still significantly lower than *T repens*. These differences were reflected in the total DM yield and in the %legume content. The plots with *T repens* had the highest clover content and the greatest DM yield, with *T. ambiguum* the lowest. Total DM yield and % clover content of the hybrids were intermediate with the BC2 greater than the BC1.

Discussion

Moisture stress is one of the most important constraints on the growth and persistence of *T. repens* (Barbour et al., 1995). *T. ambiguum* is a drought tolerant rhizomatous forage legume. This preliminary study has confirmed differences in the drought tolerance of the two species. It has shown that as the soil dried, the leaf RWC of *T. repens* decreased but in *T. ambiguum*, the leaf RWC remained higher and constant at comparable levels of soil moisture.

In the BC1 and BC2 hybrids the soil dried at the same rate as *T. repens*, but the leaf RWC decreased much more slowly and their responses were intermediate between the two parental species. They were also able to maintain a greater leaf water potential than *T. repens* at comparable soil moisture contents. This may be attributed to the fact that *T. repens* has a shallow root system whilst *T. ambiguum* has rhizomes, which improve the plants drought tolerance. The BC1 and BC2 hybrids contain both rhizome and stolon that may contribute to the greater drought tolerance of the hybrids. The yield of the BC2 hybrids, although greater than *T. ambiguum* and the BC1 was still less than *T. repens*. The yield in later years still needs to be quantified. The hybrids appear to combine the drought tolerance of *T. ambiguum* with a forage yield closer to *T. repens*. Further work is needed to improve the yield of the hybrids whilst retaining the benefits conferred by the rhizomatous growth. Research on further backcross generations is being carried out to elucidate the physiological basis of drought tolerance in these hybrids and to use molecular marker techniques to ensure the efficient introgression of the rhizomatous trait.

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Table 1- Dry matter yield of *T.repens*, *T.ambiguum* and their hybrids in plots.

Species	Legume (kg/ha)	Grass (kg/ha)	Total DM (kg/ha)	%legume
T.repens	5.92	3.46	9.39	64
T.ambiguum	0.031	2.90	2.93	1
BC1	2.39	3.46	5.90	41
BC2	4.26	3.07	7.33	58
s.e.d.	0.233***	ns	0.398(***)	

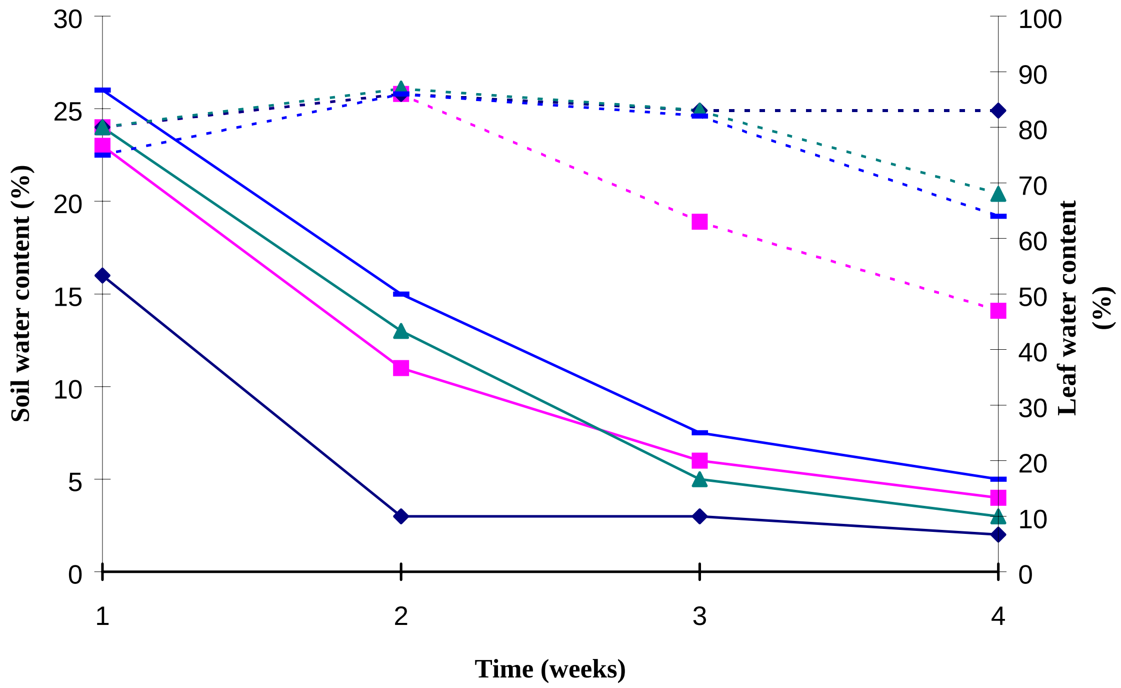

Figure 1 - Soil moisture content (%) (——) and relative leaf water content (----) of clover monocultures in bins during a four-week period without watering.
T. repens (■), *T. ambiguum* (♦), BC1 (▲) and BC2 (–).