The Effect of Summer Drought on Seasonal Changes in Taproot Reserves of Four Contrasting Alfalfa Cultivars in Tasmania, Australia

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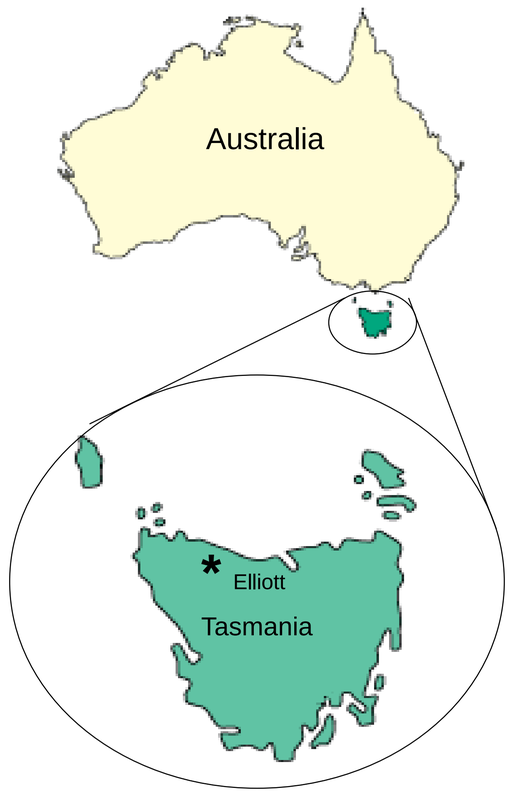
Introduction:

The strong winter dominant rainfall pattern experienced in Tasmania means summer forage production traditionally relies on irrigation. Alfalfa has been identified as a potential summer forage for the Tasmanian dairy industry due to its longer growing season when compared to perennial ryegrass grown under water limiting conditions. However, information is limited as to the effect of summer drought on taproot reserves in contrasting alfalfa cultivars. We hypothesize that summer drought will alter the cultivar effect on taproot reserves and that this effect will be dependent on drought length.



Objective:

To identify cultivar effects on taproot reserves for four alfalfa cultivars when grown with or without irrigation in Tasmania, Australia.

Methods

Field: Small plots (7×3.5 m; 4 Reps) of four contrasting alfalfa cultivars (DuPuits, FD 3; Grasslands Kaituna, FD 4.5; SARDI 7, FD 7; SARDI 10, FD 10) were established in 2006 at Elliott in north-western Tasmania. Plots were grown with or without irrigation from November 2007 to June 2008. Irrigations occurred on a 20mm water deficit (determined by estimated evapo-transpiration). Forage harvests were timed to coincide with crown bud elongation in each watering regime. Taproots were sampled at each forage harvest. Additional taproots were sampled on April 29 2008 during winter acclimation. At sampling, taproots were frozen on dry ice and lyophilized for biochemical analysis.

Glasshouse: Pots of Grassland Kaituna or SARDI 10 alfalfa (3 Reps) were exposed to a 75% water deficit or no water deficit throughout a regrowth cycle. Taproots were collected at 7 day intervals for 35 days. Samples were frozen in liquid N and ground to a fine powder with a mortar and pestle for qRT-PCR analysis, or frozen on dry ice and lyophilized for biochemical analysis.

Biochemical and qRT-PCR analysis: Taproot starch, total sugars and soluble protein concentrations were determined using procedures described by Li et al. (1996) and Cunningham et al. (1998). Analysis of taproot soluble protein pool composition was undertaken using SDS-PAGE (Laemmli, 1970). qRT-PCR analysis was undertaken with the Applied Biosystems 7900HT Fast Real Time PCR system using the standard curve method (Larionov et al. 2005) with primers specific to the high molecular weight vegetative storage protein transcript (AF530579). Data was normalized using the geometric mean (Vandesompele et al. 2002) of 3 reference genes (Elongation initiation factor, X59441; ADP-ribosylation factor, AY466444; GTP-binding protein, X79278) determined to be stably expressed in this sample set.

Statistical Analysis: Quantitative data from the field were analyzed as a split-plot in time design for each of the water regimes and data from the glasshouse were log transformed then analyzed as a complete randomized block. Qualitative results from gels were confirmed using three independent biological replicates.


Irrigated alfalfa at Elliott


Rain-fed alfalfa at Elliott

Field results

Seasonal changes in taproot carbohydrate reserves were similar in cultivars grown under both water regimes (Table 1), while there was significant cultivar by sampling date interaction on taproot soluble protein concentrations when plants were grown without irrigation (Fig. 1). Water regimes altered the seasonal changes in the taproot soluble protein pool (Fig. 2).

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Table 1. When grown with or without irrigation taproot starch decreased while total soluble sugars increased throughout the season (data averaged over 4 cultivars).

	Sugar concentration (mg/g)	Starch concentration (mg/g)
Sampling date	**Without Irrigation**	
Nov 26 2007	84.7	356
Jan 8 2008	86.5	336
Mar 10 2008	94.4	352
Arp 29 2008	105.9	289
Jun 3 2008	99.4	316
LSD (P = 0.05)	***10.8***	***31***
Sampling date	**With Irrigation**	
Nov 26 2007	79.6	352
Jan 8 2008	66	342
Feb 14 2008	87.7	282
Mar 26 2008	97	254
Arp 29 2008	105.5	173
Jun 3 2008	108.2	257
LSD (P = 0.05)	***12.5***	***43***

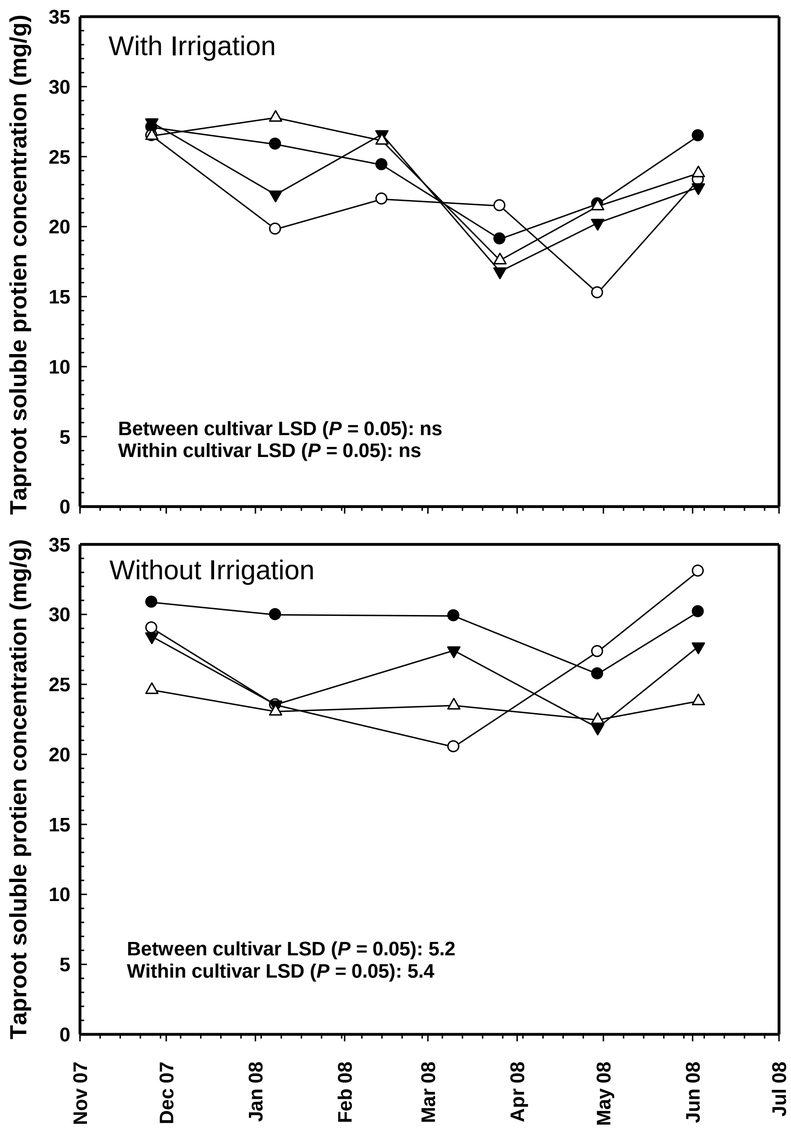

Fig 1. Without irrigation soluble protein in DuPuits (○) increased while the other cultivars (Grassland Kaituna: ●; SARDI 7 ▼; SARDI 10: △) remained stable. No differences in protein concentration were measured within or between cultivars when when alfalfa was irrigated.

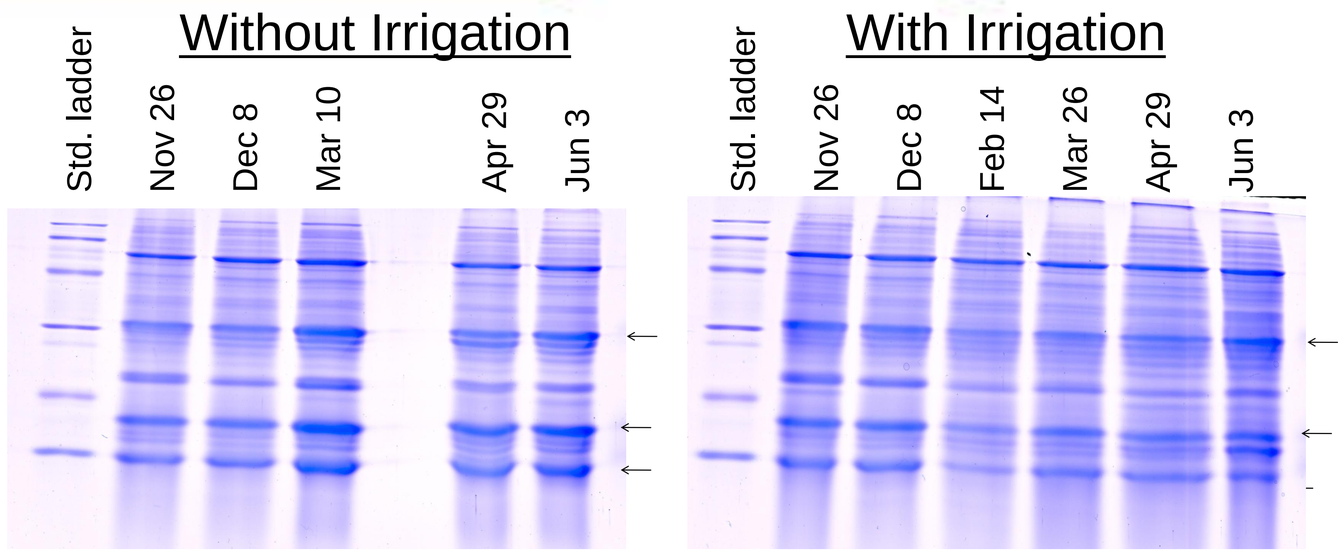

Fig 2. SDS-PAGE analysis showed that the abundance of the 3 alfalfa vegetative storage proteins (VSPs) increased in summer without irrigation, while with irrigation VSP abundance decreased over summer before increasing in fall. Arrows highlight the 32, 19 and 15 kD VSPs. The 97.4, 66.2, 45, 31, 21.5 and 14.4 kD protein standards are shown to the right. Cultivar did not affect the relative abundance of VSPs under either watering regime.

Glasshouse Results

VSP abundance increased through the regrowth period (Fig. 3). There was no difference between cultivars or water deficit. The relative abundance of transcripts encoding for the HMW VSP increased between 14 and 35 days after defoliation for both cultivars under both water regimes (Fig. 4).

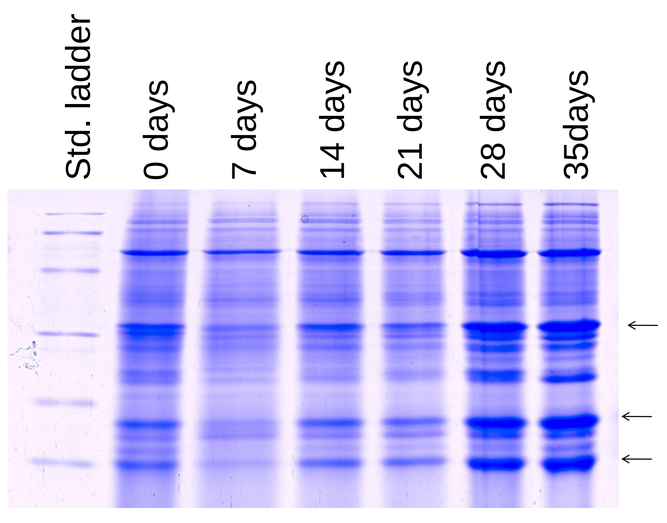

Fig 3. SDS-PAGE analysis showed that the abundance of the 3 alfalfa vegetative storage protein (VSP; Arrows highlight the 32, 19 and 17 kda proteins) increased through the regrowth period. The 97.4, 66.2, 45, 31, 21.5 and 14.4 kDa protein standards are shown to the right. There was no difference between water deficit or cultivar.

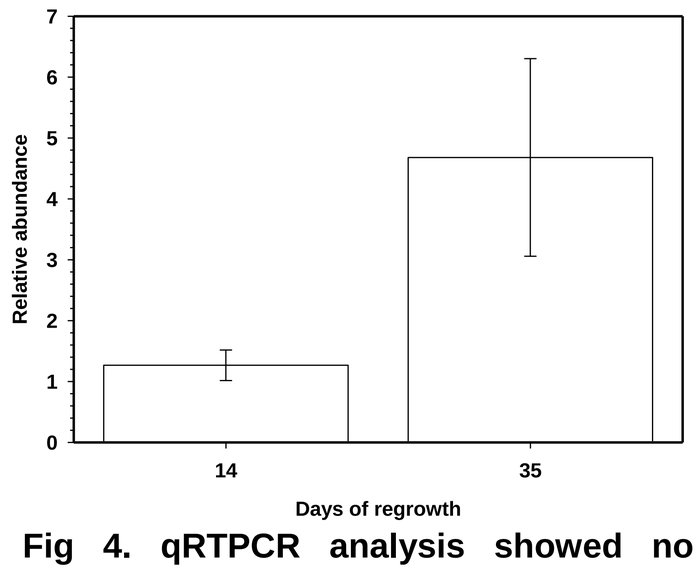

Fig 4. qRTPCR analysis showed no effect of water deficit or cultivar on the expression of the HMW VSP gene. Expression of the HMW transcript increased from 14 to 35 days after defoliation. Data averaged over cultivars and water deficits. Error bars represent the standard errors (*n* = 12).

Conclusions

Given the importance of taproot protein pools in the regrowth of alfalfa (Volenec et al. 1996) management practices should take into consideration the cultivar effect on taproot soluble protein when managing crops through a summer drought to ensure rapid recovery following re-watering. As there is no up-regulation of the HMW-VSP gene associated drought stress we propose that the increase in VSP is due to plants not fully utilising these proteins during regrowth under drought conditions.

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