

Non-response of reducing and fructosyl sugar concentrations in tall fescue during water deficit

S.G. Assuero, A. Mazzanti^{1†}, D. Barker², C. Matthew and P. Kemp

Department of Plant Science, Massey University, Palmerston North, New Zealand

¹Unidad Integrada INTA-FCA, Balcarce, Argentina

²Grasslands Division, AgResearch, Palmerston North, New Zealand

Abstract

Reducing and fructosyl sugar concentrations of two contrasting tall fescue (*Festuca arundinacea* Schreb.) cultivars, El Palenque (EP) of Temperate origin, and Maris Kasba (MK) of Mediterranean origin, were compared in response to water deficit in a glasshouse experiment. Four watering treatments were applied from 17 October to 12 November 1996: control (fully irrigated), S1 (80% of control), S2 (65% of control) and S3 (50% of control). At the end of this period, two tillers per cultivar per pot were sampled at dawn to determine relative water content (RWC). Reducing and fructosyl sugar contents were determined at midday on the youngest fully expanded leaf of two tillers per cultivar per pot. Interactions between cultivars and water treatments for the variables studied were not significant. Differences in RWC were not significant between cultivars, but were significant among water treatments ($P < 0.05$). Reducing sugar content ($\mu\text{mol glucose g}^{-1}$ FW) was higher ($P < 0.05$) for EP (47.46) than for MK (38.23). The same result was observed for fructosyl sugars (EP = 22.37 vs. MK = 9.8). There was no evidence that water deficit increased reducing or fructosyl sugar content ($P > 0.05$). None of the sugars measured made a significant contribution to the osmotic adjustment of these tall fescue cultivars under water stress conditions, indicating that other compounds were involved in osmotic adjustment.

Additional key words: tall fescue, reducing sugars, fructosyl sugars, water deficit, Mediterranean genotype

Introduction

Forage species exhibit a decrease in osmotic potential as a consequence of the accumulation of solutes under drought (Sambo, 1981; Barker *et al.*, 1993). The composition of these solutes is largely unknown. However, among the solutes that accumulate in grasses of temperate origin are fructans (Pontis and del Campillo, 1985). It was found that under drought conditions fructans were depolymerised in wheat (*Triticum aestivum* L.) stems after anthesis, promoting osmoregulation and turgor maintenance (Virgona and Barlow, 1991), although, in tall fescue (*Festuca arundinacea* Schreb.) fructan seems to contribute only indirectly to osmotic adjustment (Spollen and Nelson, 1994).

The objective of this experiment was to compare the concentration of reducing and fructosyl sugars of the youngest fully expanded leaf of two contrasting tall fescue genotypes when subjected to water stress.

Materials and methods

Two endophyte-free tall fescue cultivars El Palenque (EP), of temperate origin from INTA Pergamino, Argentina; and Maris Kasba (MK), of Mediterranean origin from INRA, France, were used. Seeds were germinated and seedlings transferred to root-trainers filled with vermiculite, and irrigated three times a week with 0.5M Hoagland's solution (Hoagland and Arnon, 1938). Plants were grown in a glasshouse at Unidad Integrada INTA-FCA Balcarce (37° 45' S), under the ambient photoperiod. Average maximum temperature was 39.9°C and average minimum temperature 11.9°C. On 8 July 1996 the main tiller of each plant was transplanted to pots of 300 x 370 x 320 mm, filled with friable loam soil (A horizon) with 29.9 ppm of phosphate, 47.3 ppm of nitrate and 6.4% organic matter. Each pot contained four rows of five tillers with the two cultivars arranged in alternate rows; EP was defoliated to ensure similar leaf area as MK. Pots were irrigated daily and once a week received 250 ml of 0.5M Hoagland's solution. On 17 October four water treatments were imposed; namely: control = 1,000 ml water day⁻¹ pot⁻¹;

[†] now deceased

S1 = 800 ml water day⁻¹ pot⁻¹; S2 = 650 ml water day⁻¹ pot⁻¹ and S3 = 500 ml water day⁻¹ pot⁻¹.

On 12 November the youngest fully expanded leaf of two tillers per cultivar per pot was sampled at 5.30 AM to determine RWC (Turner, 1981) and at midday to determine the reducing and fructosyl sugar concentration. Extracts used for the determination of sugar concentration were prepared by boiling the fresh sample twice in alkaline water (2 drops of 0.1M NH₃ solution per 125 ml water) for 10 minutes. The approximate ratio of water to sample fresh weight for each partial extraction was 1 ml:0.1 g. Reducing sugars (glucose and fructose) were determined by Somogyi-Nelson's method (Somogyi, 1952) and fructosyl sugars (free fructose, sucrose and fructans) by Kulka's method (Kulka, 1956). The contribution of fructosyl sugars to the osmotic adjustment was calculated from the concentration of fructosyl sugars in the total volume of the tissue at 100% relative water content and assuming that 40 μmol g⁻¹ H₂O = 0.1 MPa (Munns and Weir, 1981). Osmotic potential (Ψ_o) was measured at midday on the youngest fully expanded leaf of two tillers per cultivar per pot using a 5500 Wescor Vapor Pressure Osmometer as described by Turner (1981). Osmolalities (mmol. kg⁻¹) were converted into osmotic pressure (MPa) by multiplying by a factor of 2.48 x10⁻³ according to van't Hoff's equation (Hohl and Schopfer, 1991).

A split plot design was used in which watering treatments were main plots and cultivars were subplots. A weighted ANOVA was used for the analysis of the fructosyl sugar concentration data because of variance heterogeneity among water treatments. The reciprocal of the error variances of the individual by water treatment analysis were used as weights for the observations. RWC data were arcsin square root transformed for statistical analysis but untransformed means are given in the results. Means were separated using the LSD (Steel & Torrie, 1980) at P = 0.05.

Results and discussion

There were no significant interactions between cultivars and water treatments for any of the variables considered. The RWC decreased significantly with decreased water application (P < 0.05, Table 1).

The concentrations of reducing and fructosyl sugars were 24% and 127% respectively higher for EP than for the Mediterranean cultivar MK (P < 0.05, Table 1). Eagles (1967) compared the simple sugar and fructan concentrations of two populations of cocksfoot (*Dactylis glomerata* L.), one from a North European environment (Norway) and the other from a Mediterranean

environment (Portugal) in response to different temperatures. In contrast with our results, he found no significant difference in simple sugar content between populations. Under the elevated temperatures registered in the glasshouse, the temperate cultivar EP had a significantly greater relative growth rate, estimated as the ratio between net growth rate (g plant⁻¹ d⁻¹) and final plant size (g plant⁻¹), than the Mediterranean cultivar (e.g., EP = 0.53% DW d⁻¹ and MK = 0.40% DW d⁻¹, P = 0.02), in agreement with earlier results (Morgan, 1964). In view of this higher growth rate of EP, a lower concentration of sugars available for conversion to fructans might have been expected (Eagles, 1967), and the reason for the present results was unclear.

In the present study no systematic trend was observed in reducing sugar concentration in response to water stress (Table 1). The fructosyl sugar concentration in S1 tended to be lower than in the control, while S2 and S3 tended to give higher concentrations (Table 1), however,

Table 1. Relative water content (RWC), and reducing and fructosyl sugar concentration of the cultivars El Palenque (EP) and Maris Kasba (MK) for the water treatments studied (Control, S1 = 80% of control, S2 = 65% of control and S3 = 50% of control).

Treatments	RWC ¹ (%)	Reducing sugars (μmol glucose g ⁻¹ FW)	Fructosyl sugars (μmol fructose g ⁻¹ FW)
Cultivar (C)			
MK	90.5 (74.0)	38.23 b ²	98.43 b
EP	91.9 (74.3)	47.46 a	223.66 a
SEM	(0.8)	2.42	11.99
Water (W)			
Control	95.7 (79.4)a	43.36	161.59
S1	93.1 (76.9)ab	37.82	106.17
S2	89.5 (71.5)bc	45.55	174.51
S3	86.5 (68.8)c	44.67	201.91
SEM	(2.2)	6.35	22.12
Interaction (C*W)	P = 0.31	P = 0.57	P = 0.22

¹ Data within brackets are arcsin square root transformed percentages.

² Letters indicate groupings of means that are significantly different at P < 0.05 within cultivar or water treatments.

the variability was such that no definitive interpretation can be placed on these trends. The tendency for a higher sugar concentration found in the control than in S1 could have been considered the result of a higher photosynthetic rate in non-stressed plants.

The contribution of fructosyl sugars to the osmotic adjustment did not differ significantly among the water treatments and ranged from 0.3 MPa to 0.6 MPa. This finding was consistent with Spollen and Nelson (1994) in that fructosyl sugars did not seem to be involved directly in osmotic adjustment. Moreover, it should be noted that in the above calculation the osmotic contribution might have been overestimated because the differential contribution of fructose polymers of different molecular weight was not taken into account. That the osmotic potential of both cultivars became more negative with the increase in water stress (e.g., C = -1.9 MPa, S1 = -2.1 MPa, S2 = -2.4 MPa and S3 = -2.7 MPa, $P < 0.05$) and the contribution of the fructosyl sugars to the osmotic potential was not different between water treatments (e.g., C = 26%, S1 = 16%, S2 = 21% and S3 = 22%, $P > 0.05$), is further confirmation that solutes other than the sugars measured were involved in the osmotic adjustment.

Conclusions

Under the environmental conditions of this experiment, the concentration of both reducing and fructosyl sugars were higher in the temperate cultivar EP than in the Mediterranean cultivar MK. None of the sugars measured made any significant contribution to the osmotic adjustment of these tall fescue cultivars under water stress conditions, indicating that other osmotic compounds were involved.

Acknowledgements

This work was supported by a NZODA scholarship and the Unidad Integrada INTA Balcarce. Thanks to Dr. François Gastal for supply of the seed, to Dr. Garry Latch for the seed treatment, to Ing. Agr. Patricio Miravé, Dr. Elsa Camadro and Dr. Carlos Barassi for the facilities provided, to Dr. Jorge Tognetti, Lic. Alejandra Equiza and Ing. Agr. Mabel Casanovas for assistance with the carbohydrate analyses, and to Ms Silvia Larraburu for technical assistance.

References

- Barker, D.J., Sullivan, C.Y. and Moser, L.E. 1993. Water deficit effects on osmotic potential, cell wall elasticity, and proline in five forage grasses. *Agronomy Journal* **85**, 270-275.
- Eagles, C.F. 1967. Variation in the soluble carbohydrate content of climatic races of *Dactylis glomerata* (Cocksfoot) at different temperatures. *Annals of Botany* **31**, 645-651.
- Hoagland, D.R. and Arnon, D.I. 1938. The water method of growing plant without soil. *California Agricultural Experiment Station Circular* **347**, 1-16.
- Hohl, M., and Schopfer, P. 1991. Water relations of growing maize coleoptiles. *Plant Physiology* **95**, 716-722.
- Kulka, R.G. 1956. Colorimetric estimation of ketopentoses and ketoexoses. *Biochemical Journal* **63**, 542-548.
- Morgan, D.G. 1964. The eco-physiology of Mediterranean and north temperate varieties of tall fescue. *Outlook on Agriculture* **4**, 171-176.
- Munns, R. and Weir, R. 1981. Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits at two light levels. *Australian Journal of Plant Physiology* **8**, 93-105.
- Pontis, H.G. and del Campillo, E. 1985. Chapter 5: Fructans. In *Biochemistry of storage carbohydrates in green plants* (eds. P.M.Dey and R.A. Dixon), pp. 205-227. Academic Press, New York.
- Sambo, E.Y. 1981. Osmotic adjustment as a mechanism of drought resistance in crop and forage species. *Journal of Science and Technology (Malawi)* **2**, 21-37.
- Somogyi, M. 1952. Notes on sugar determination. *Journal of Biological Chemistry* **195**, 19-23.
- Spollen, W.G. and Nelson, C.J. 1994. Response of fructan to water deficit in growing leaves of tall fescue. *Plant Physiology* **106**, 329-336.
- Steel R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. A biometrical approach, 2nd Edition. McGraw Hill Book Company. 633 pp.
- Turner, N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil* **58**, 339-366.
- Virgona, J.M. and Barlow, E.W.R. 1991. Drought stress induces changes in the non-structural carbohydrate composition of wheat stems. *Australian Journal of Plant Physiology* **18**, 239-247.