Leaves of High Yielding Perennial Ryegrass Contain Less Aggregated Rubisco than S23

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Introduction Breeding diploid perennial ryegrass for improved dry matter yield under nitrogen-limiting conditions has reduced the nitrogen (N) concentration of the herbage (Wilkins *et al*., 2003). Reduced N concentration in the ruminant diet is one potential way to reduce losses of N to the environment by reducing the amount of N that animals excrete. The underlying physiological basis of this increased N-use efficiency in ryegrass was investigated.

Materials and methods Leaf samples were taken from the third harvest year (2004) of a field plot trial with 4 replicate fully randomised blocks containing two perennial ryegrass varieties that had varied consistently in N concentration throughout the first two harvest years (Wilkins *et al*., 2003): Ba13582, which had the lowest mean N concentration over all harvests, and S23, which had the highest. Ba13582 produced significantly more dry matter than S23 in both these harvest years (2002 and 2003). Ten fully expanded leaves from each plot were frozen in liquid N_2 and stored at -80^{0}C. Samples were ground to a fine powder and protein was extracted by grinding in a neutral buffer (0.1 M HEPES, pH 7.5, 1 mM EDTA, 2 mM DTT, 0.1% Triton X-100, 1 mM PMSF, 1 μM E64) at a ratio of 25 ml per g dry weight. After centrifugation (5 min at 10,000g_{av}), protein contents of the supernatants were determined (Bradford, 1977) while protein separation was achieved by denaturing gel electrophoresis (Laemmli, 1970). Gels were loaded with 10 μg protein per sample track plus molecular weight standards. They were stained with Coomassie blue and analysed by densitometry (BioRad GS710 equipped with Qantity One software, BioRad UK, Hemel Hempstead). Analyses of variance were carried out using GENSTAT.

Results Densitometric analysis of the major leaf protein bands of Ba13582 and S23 did not reveal significant differences between the varieties in concentration of the large and small subunits of Rubisco (Table 1). However, Ba13582 contained less than half the amount of high molecular weight polypeptide (~205 kDa) that was present in S23. This 205 kDa polypeptide is typical of non-heat dissociable, aggregated Rubisco subunits.

Table 1 Densitometric analysis (OD x mm^2) of Rubisco protein bands in leaf protein extracts from Ba13582 and S23 resolved by denaturing electrophoresis

Variety	Large subunit	Small subunit	Aggregated
Ba13582	31.3	10.4	2.9
S23	24.8	8.0	6.9
s.e.d.	2.23	1.18	0.56
p	NS	NS	0.006

NS, not significant at p=0.05

Conclusions Since aggregated Rubisco is unlikely to function in capturing CO_2 from the atmosphere, this result suggests a possible mechanism for the superior N-use efficiency of Ba13582. The *in vivo* significance of aggregated rubisco is unclear. It may represent an N storage pool, to which Ba13582 partitions less assimilated N than S23. Alternatively, it may indicate protein damage. In either case, Ba13582 would be predicted to achieve efficient photosynthesis with less protein N than S23. Families derived from Ba13582 are currently being used at IGER for genetic mapping. If our hypothesis is correct, it should be possible to identify quantitative trait loci that control both N-use efficiency and the amount of aggregated Rubisco.

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