PVI Perennial Ryegrass Improvement Program

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EXECUTIVE SUMMARY

During this project and its predecessors a number of synthetic cultivars of perennial ryegrass have been developed for release into a range of Australian environments. The final phase of this project (1999 – 2001) has focussed on the breeding and commercial development of drought tolerant perennial ryegrass cultivars based on germplasm of Mediterranean origin.

A synthetic cultivar (AVH4) has been selected, evaluated and is currently under seed bulk up in conjunction with a commercial partner. This cultivar has the potential to increase the productivity and persistence of perennial ryegrass pasture in lower rainfall environments throughout temperate Australia. The likely benefits of this cultivar over the standard cultivar Victorian were assessed using the computer simulation GrazFeed with merino cross wethers. In a target environment (Balmoral) the dry matter yield advantage in winter of AVH4 over Victorian of 0.66 t DM/ha predicted to raise the carrying capacity of the pasture (potential stocking rate) by 45% from 10 to 14.5 wethers per hectare. Similar results were obtained in spring were a 65% increase in carrying capacity was predicted.

Physiological experiments were undertaken under controlled environment conditions to assess the physiological basis of the improved persistence of Mediterranean perennial ryegrass genotypes in low rainfall environments. An analysis of some of the potential physiological mechanisms indicated that summer dormancy, increased rooting growth and the ability to regulate photosynthesis/transpiration under drought stress are all involved in the adaptation of this germplasm to summer drought. Further elucidation of these mechanisms and the development of molecular markers for these traits will allow them to be incorporated into a broader range of germplasm more quickly than with traditional breeding methods.

The commercial use of high yielding and persistent perennial ryegrass cultivars such as AVH4 in lower rainfall environments has the potential to greatly improve the persistence and productivity of perennial ryegrass based pastures in these areas which are traditionally sown to the unimproved ecotype Victorian perennial ryegrass.

CONTENTS

1. BACKGROUND

Perennial pasture grasses are an important component of the temperate pasture of southern Australia. In 1993-1994 there were over 29.5 million hectares of pasture in Australia (ABS 1996). The main perennial grass species sown in the higher rainfall region (>550 mm annual rainfall) of southern Australia are the introduced species perennial ryegrass, tall fescue, phalaris and cocksfoot. Whilst each of these species has key characteristics that contribute to improved productivity of grazing systems and is adapted to different environmental conditions, there is significant variation within each species and marked overlap in the zones of adaptation. In general, pasture in the 450 – 650 mm rainfall zone of southern Australia has only a low proportion of perennial grass species present (Quigley 1991) due to problems with the establishment and persistence of perennial grass species in these environments. This is particularly the case with perennial ryegrass where there has been little attention focussed on breeding improved cultivars for marginal environments.

In 1989 a project was commenced to breed perennial ryegrass cultivars adapted to a range of Australian conditions including the zone that is considered to be marginal for perennial ryegrass persistence. Perennial ryegrass is still a favoured species in these regions due to its ease of establishment and potential to produce high yields of nutritious forage. This component of the project focussed on the development of selections based on germplasm of Mediterranean origin. Germplasm from the Mediterranean has been widely used in the development of a range of pasture grass and legume cultivars adapted to lower rainfall regions in temperate Australia but not in perennial ryegrass breeding programs. The drought tolerance and persistence of Mediterranean accessions of perennial ryegrass under Australian conditions was first measured by Silsbury (1961) and the cultivar Medea was released as a result of this evaluation. However, Medea was not commercially successful due to problems with seed yield and threshability. The program described in this report commenced with the evaluation of accessions from a wide range of genetic backgrounds at Balmoral in the central west of Victoria and was followed by half-sib family evaluation in a range of target environments in Victoria, South Australia, Western Australia, NSW and Tasmania (a full description of this program was given in the final report of the predecessor to this project DAV067 (Cunningham 1996)).

This report describes the completion of this project during the years 1999 – 2001 and includes information on the following aspects of the breeding of drought tolerant perennial ryegrass germplasm:

- Agronomic performance of the synthetic cultivar AVH4
- Progress with seed multiplication and certification
- Progress with the commercial development of AVH4
- A description of the possible physiological mechanisms of persistence in this germplasm
- Computer simulation of the potential animal production benefits from the use of this germplasm compared to the standard cultivar Victorian
- A list (and some examples) of the scientific publications relating to this project.

1.1 Agronomic performance of the synthetic cultivar AVH4

AVH4 is a synthetic perennial ryegrass cultivar of North African parentage that was chosen for commercial release based on the results of cultivar trials of parents and progeny in a range of target environments. Following seed increase in 1998/99 and 1999/2000, further evaluation experiments to compare the synthetic cultivar with existing commercial cultivars were sown in autumn 2000 at Balmoral, Bulart and Hamilton Victoria. Each trial was sown as 5 x 1.5 m plots replicated six times in a latinised row-column design. Perennial ryegrass seed was sown in monoculture at 15kg/ha with seeding rates adjusted for differences in seed size and germination between cultivars.

All of these trials have established well and herbage yield data for 2000 and the first half of 2001 are presented in tables 1 – 3. The coded entries AVH3, AVH5 and AVH6 are sister lines of the cultivar being developed for commercial release (AVH4). AVH4 is performing well compared to Victorian, the main cultivar sown in these drier environments. However, the data are only from early stages of the evaluation and the real benefits of the increased persistence of the Mediterranean germplasm are not expected to be realised until years 3 and 4 of evaluation.

These trials are continuing to be harvested and measured for herbage yield with support from the commercial partner for the germplasm and the full data will form part of the companies marketing package for AVH4.

Table 1: Establishment and yield summary for Hamilton perennial grass evaluation trial.

Cultivar	Establishment (Plants/m^2)	22/09/00	14/11/00	Total 2000	18/5/01	13/7/01	16/8/01	16/10/01	4/12/01
Avalon	423	2.98	3.54	6.52	3.22	2.49	1.90	4.17	3.76
AVH 3	402	2.90	3.33	6.22	2.65	2.33	1.56	4.43	3.19
AVH 4	341	2.89	3.11	5.99	**3.63**	**2.63**	1.51	3.85	3.27
AVH 5	410	2.96	3.29	6.22	2.58	2.37	1.75	4.11	3.56
AVH 6	429	2.98	3.38	6.35	3.03	2.50	1.91	4.28	3.22
Boomer	452	3.22	3.21	6.43	3.15	2.57	2.06	3.66	2.68
Camel	449	2.73	3.16	5.89	2.26	2.29	1.65	4.52	3.27
Ellett	493	3.03	3.36	6.38	2.87	2.41	2.32	4.27	3.36
Fitzroy	580	**3.18**	3.48	**6.67**	3.18	2.50	2.44	3.92	2.97
G. Impact	465	3.05	3.34	6.40	3.09	2.40	2.45	4.01	3.65
Meridian	351	3.06	3.17	6.23	2.83	2.51	2.11	4.12	3.36
Roper	376	2.86	2.98	5.85	2.87	2.38	1.52	4.17	2.94
Samson	429	2.96	**3.61**	6.57	2.89	2.41	2.21	3.78	3.10
Vedette	437	3.09	3.42	6.51	3.03	2.46	2.05	3.94	3.40
Victorian	467	2.97	3.33	6.29	2.57	2.38	1.89	4.77	3.23
Yatsyn1	393	2.80	3.41	6.22	2.95	2.49	2.02	4.18	3.43
LSD (P=0.05)	67.3	0.212	0.251	0.351	0.628	0.201	0.277	0.522	0.451
CV%	13.5	5.1	6.1	4.4	17.2	6.3	11.2	10.3	11.9

Table 2: Establishment and yield summary for Balmoral perennial grass evaluation trial.

Cultivar	**Establishment (Plants/m^2)**	**22/11/00 (transformed)**	**22/11/00**	**12/6/01**	**3/7/01**	**22/8/01**	**24/9/01**	**8/11/01**	**14/12/01**
Avalon	351	8.0	3.04	1.84	1.95	1.56	2.92	2.68	4.02
AVH 3	357	7.8	2.53	1.57	1.91	1.47	2.53	2.58	3.59
AVH 4	272	7.8	2.47	1.85	**2.03**	1.76	**3.03**	2.64	3.84
AVH 5	334	7.9	2.74	1.56	1.95	1.42	2.68	2.35	3.70
AVH 6	368	8.0	3.05	1.70	1.89	1.55	2.71	2.36	3.54
Banks	345	8.1	3.27	1.77	1.91	1.55	2.52	2.38	3.72
Boomer	337	8.0	3.08	1.45	1.87	2.52	2.01	2.11	3.25
Bronsyn	345	8.1	**3.40**	2.17	1.99	1.71	2.50	2.30	3.70
Camel	376	7.9	2.63	1.36	1.88	1.51	**3.03**	2.71	3.12
Dobson	331	8.1	3.44	1.79	1.91	1.49	2.47	2.35	3.55
Ellett	382	8.1	3.26	1.45	1.86	1.32	2.60	2.43	3.41
Fitzroy	439	8.1	3.26	1.93	2.01	2.07	2.82	2.42	3.50
G. Impact	340	8.1	3.20	**2.32**	2.01	1.79	2.84	2.53	**4.42**
Meridian	327	8.0	3.00	1.59	1.99	2.23	3.00	2.61	3.72
Roper	320	7.9	2.70	1.24	1.98	2.13	2.46	2.17	3.24
Samson	368	8.1	3.34	1.53	1.91	1.52	2.14	2.14	3.82
Vedette	387	8.1	3.40	1.75	1.82	1.34	2.39	2.01	3.53
Victorian	400	8.1	3.16	1.19	1.73	1.08	2.30	2.20	3.40
Yatsyn1	323	8.1	3.18	1.31	1.91	1.50	2.58	2.40	3.89
LSD (P=0.05)	54.3	1.08	-	0.401	0.142	0.447	0.631	0.462	0.513
CV%	11.4	1.4	-	18.4	5.9	21.0	18.5	14.9	11.6

Table 3: Establishment and yield summary for Bulart perennial grass evaluation trial.

Cultivar	Establishment (Plants/m^2)	5/10/00	6/11/00 (transformed)	6/11/00	12/12/00	Total 2000	7/5/01	26/6/01	24/8/01	10/10/01	21/11/01
AVH4	301	2.35	8.1	3.32	2.82	8.58	**3.09**	**3.11**	4.49	**4.51**	4.08
AVH 5	316	2.39	8.2	3.49	2.81	8.78	2.51	2.50	3.03	3.56	4.07
AVH6	350	2.76	8.1	3.31	2.85	8.90	2.22	2.19	3.59	4.15	4.44
Boomer	340	**3.17**	8.1	3.29	2.52	8.96	2.14	2.15	**5.34**	3.10	3.92
Camel	335	2.51	8.3	3.84	2.66	9.13	2.19	2.19	3.14	4.06	3.73
Ellett	349	2.64	8.2	3.77	3.19	9.70	2.44	2.41	3.09	4.12	3.84
Fitzroy	416	3.04	8.3	3.90	3.04	10.01	2.75	2.77	4.49	3.60	3.98
G. Impact	361	2.73	8.2	3.61	3.16	9.56	3.07	3.05	4.15	4.00	4.19
Meridian	317	2.86	8.1	3.45	2.80	8.98	2.54	2.53	4.52	3.78	3.96
Roper	340	2.69	8.1	3.39	2.49	8.69	2.03	2.02	4.14	3.22	3.73
Vedette	340	2.77	8.2	3.72	2.99	9.48	2.75	2.78	3.95	4.16	3.98
Victorian	381	2.57	8.3	**4.11**	**3.25**	**10.13**	2.37	2.41	3.25	4.12	3.97
LSD (P=0.05)	50.0	0.39	1.09	-	0.337	1.052	0.347	0.352	0.465	0.468	0.356
CV%	11.7	12.2	1.7	-	8.5	8.7	10.3	10.2	9.0	9.0	7.3

1.1.1 Progress with seed multiplication

All of the Mediterranean synthetics were bulked to SynII stage during this project (approximately 10kg seed of each). The selected synthetic, AVH4 has been further multiplied to SynII stage and the production of commercial quantities of seed is currently under way in conjunction with Vicseeds Pty Ltd.

1.1.2 Commercial development

AVH 4 was developed from the DAV 94 Drought tolerant ryegrass sub-project BP1/1. In December 1999 AVS on behalf of DNRE, MLA and DRDC accepted a tender submitted by Vicseeds Pty Ltd to become the commercial licensee fro AVH 4. A draft technical services agreement and licence option has been developed and after considerable negotiation has been signed. A licence option for AVH 4, the most promising synthetic cultivar is currently being drafted, a copy of which will be sent to partners upon signing. A copy of the "Technical Services Agreement and Licence Option" between Agriculture Victoria Services and Vicseeds Pty Ltd is attached to this report as Appendix 1.

Pre-basic AVH 4 will be seed increased at PVI Hamilton in 2002. Seed will be handed over to Vicseeds in January 2003 for the commencement of basic and commercial seed multiplication. Plant Breeders Rights protection will be applied for in 2002 with the DUS trial run in 2002 and 2003. It is expected that commercial release will occur in autumn 2004.

AVH 4 is currently included in regional trials being conducted by the Cultivar Development Unit to collate additional performance information for use in the commercial release of AVH 4.

2. Physiological Studies

2.1 Photosynthesis

The productivity and sustainability of the grazing industries of Australia are limited by the susceptibility of perennial ryegrass to annual summer drought. The evaluation of drought tolerance in the field is time consuming and expensive. Hence the need exists to develop mechanistic tests of drought-tolerance strategies among perennial ryegrass genotypes. In this project perennial ryegrass genotypes of Mediterranean origin have been shown to be more tolerant of drought than commercially available cultivars that are derived from Australian or New Zealand germplasm (Anderson *et al.* 1999).

The ability of plants to maximise photosynthetic capacity and water use efficiency is important in maintaining growth and survival under conditions of stress. Water stress has been shown to decrease photosynthesis in perennial ryegrass due to reductions in leaf area, increased stomatal resistance and a decrease in the quantum efficiency of photosynthesis, depending on the rate of onset and the extent of water stress (Jones *et al* 1980). Differences in the quantum efficiency of perennial ryegrass genotypes have been observed with Danish populations of perennial ryegrass reaching light saturation at lower light intensities than populations from Algeria (Eagles 1967). We measured the photosynthetic characteristics of two contrasting perennial ryegrass genotypes; one of North African origin from a population that has previously been shown to survive well through summer drought (Anderson *et al.*

1999) and another from the cultivar Aurora of Swiss origin. Aurora has previously been shown to be susceptible todrought in Mediterranean France (Volaire *et al.* 1998).

Under well watered conditions the north African genotype was shown to have a higher maximum rate of photosynthesis (24 _mol/m2/s) than the genotype from cv. Aurora (16 _mol/m2/s) (See McFarlane *et al.* 2001 in Appendix 2). The plants that were used to develop the cultivar Aurora were originally derived from a collection from the Zurich uplands in Switzerland. This difference in quantum efficiency - photosynthetic rate per unit irradiance at high irradiance - between diverse perennial ryegrass genotypes is consistent with previously published results. The ecological significance of these results is unclear but it has been hypothesised that differences in photosynthetic activity may be related to the increased cool-season growth and water-soluble carbohydrate storage that may be observed in ecotypes and cultivars of forage grasses adapted to Mediterranean climates (Arcioni *et al.* 1985).

The rate of photosynthesis of leaves of both genotypes was greatly reduced by imposition of water stress with the North African genotype having a maximum assimilation rate of 2 _mol/m2/s and Aurora 6 _mol/m2/s. Similar large reductions in the photosynthetic capacity have been observed in perennial ryegrass swards in drought conditions (Jones *et al.* 1980). Whilst drought has been shown to reduce the quantum efficiency of perennial ryegrass swards it is also possible that the effects that we observed were magnified due to a reduced ability of a plants to acclimatise to drought under controlled conditions (Jones *et al.* 1980). However, it is also possible that a reduction in the photosynthetic rate of the North African genotype was associated with decreased stomatal conductivity and a general reduction in the activity of this summer-dormant genotype under drought conditions.

This improved photosynthetic control under drought conditions, summer dormancy and improved root growth of North African genotypes relative to some other drought susceptible cultivars (Guthridge *et al.* 2001, Smith *et al.* unpublished) are all likely to lead to improved persistence under drought conditions, further research is required to fully elucidate these mechanisms.

3. Scientific publications containing results from this project (1999 – 2001)

3.1 Journal articles

Anderson, M.W., Cunningham, P. J., Reed, K. F. M. and Byron A (1999) Perennial grasses of Mediterranean origin offer advantages for central western Victorian sheep pasture. *Australian Journal of Experimental Agriculture* **39** 275 – 284.

Reed, K.F.M., Culvenor, R.A., Jahufer, M.Z.Z., Nichols, P., Smith, K.F., and Williams R. (2001). Progress and challenges: Forage breeding in temperate Australia. *In* Molecular Breeding of Forage Crops (G. Spangenberg, ed.) (Kluwer Academic Publishers, Dordrecht, The Netherlands). pp 303-316*.*

3.2 Conference papers

Guthridge, K.M., Jones, E.S., Smith, K.F., McFarlane, N.M. and Forster, J.W. (2000). Mapping drought tolerance associated genes in perennial ryegrass. 2nd International

Symposium *Molecular Breeding of Forage Crops,* Lorne & Hamilton, Vic., 19-24th November 2000. Abstracts, p. 81.

McFarlane , N.M., Smith, K.F., Jones, E.S., Guthridge, K.M. and Forster, J.W. (2000). Mapping QTLs for photosynthetic characteristics in *Lolium perenne* to improve water-use efficiency. 2nd International Symposium *Molecular Breeding of Forage Crops,* Lorne & Hamilton, Vic., 19-24th November 2000. Abstracts, p. 83.

Reed KFM, Smith KF, Jahufer Z and Anderson MA (2000). New forage cultivars developed by Agriculture Victoria for Australian Animal Industries. *Animal Science in Agriculture Victoria*, The Agriculture Victoria Animal Institute Group Research Conference, Melbourne, May 17-18, 2000*. P 1-22*

Reed KFM and Smith KF (2001) Adaptation of Perennial ryegrasses to a long dry summer. Proceedings 23rd EUCARPIA Fodder Crops and Amenity Grasses Section Meeting, Breeding for stress tolerance Terceira, Azore Islands, Oct 1-4th 2000. (in Press).

3.3 Intellectual property

This project has developed both generic and specific intellectual property.

Generic intellectual property

Generic intellectual property regarding the role and performance of Mediterranean germplasm has been developed during this project. This IP has been promoted to interested parties through a range of scientific and technical publications. The adoption of this IP in other breeding programs may lead to the development of further new cultivars for the target environment that could be of further benefit to industry.

3.4 Specific intellectual property

This project also generated specific intellectual property in the form of elite perennial ryegrass (plants and seeds) selected in target environments for agronomic performance and disease resistance. The cultivar AVH4 is being released to industry through a license agreement (managed by Agriculture Victoria Services) with the commercial partner paying a royalty to the product developers based on seed sales. All co-investors during product development share in royalties according to the NPICC formula with equity determined based on inputs. Any future releases or development of non-commercial germplasm from this project will be released according to similar guidelines following negotiation with all stakeholders.

4. Industry Implications of this Research

The development of productive, drought tolerant perennial ryegrass cultivars has the potential to greatly increase the profitability and sustainability of perennial ryegrass pasture in the 500 – 650 mm rainfall environments of temperate Australia. In order to estimate the potential benefits of these improved cultivars the decision support package GrazFeed was used to predict the animal production that would be achieved through grazing the Mediterranean cultivars compared to Victorian perennial ryegrass which is the most prevalent cultivar in these environments and is marketed based on its drought tolerance and persistence.

GrazFeed simulation: The yields of AVH4 and Victorian PRG at two dates (winter and spring) were used in GrazFeed to evaluate the performance (daily intake and growth rates) of merino-cross wethers and the stocking rate that could be supported by the two pastures at each date. These data were derived from second year sward trials where both cultivars had experienced one summer.

Temperatures and pasture growth rates typical for each date were used in the simulations. One cultivar (AVH4) was assigned the typical pasture growth rate for the date, and the other (Victorian) had pasture growth rate calculated from the typical growth rate, based on the ratio of DM yields of the two cvv. Digestibility of pasture was assumed to be 80% and CP to be 16% for all tests.

The dry matter yield advantage in winter of AVH4 over Victorian of 0.66 t DM/ha (Table 4) was predicted to raise the carrying capacity of the pasture (potential stocking rate) by 45% from 10 to 14.5 wethers per hectare. Similar results were obtained in spring were a 65% increase in carrying capacity was predicted.

Whilst evaluation of animal performance is required to validate these predictions the values do give an indication of using well adapted perennial ryegrass germplasm in these environments.

Table 4. Daily intakes and animal growth rates predicted by GrazFeed for merino-cross wethers grazing either Victorian perennial ryegrass or a synthetic line (AVH4) selected for drought tolerance, based on pasture yields measured at Balmoral, SW Victoria.

	Winter		**Spring**	
	AVH4	Victorian	AVH4	Victorian
Yield (t DM/ha)	1.85	1.19	3.03	1.80
Pasture growth rate (kg/ha/d)#	37	24	100	60
Intake (kgDM/head/d)	1.7	1.6	1.8	1.8
Animal growth rate (g/head/d)	147	127	171	163
Stocking rate (head/ha)+	14.5	10	37.0	22.0

Pasture growth rate values for AVH4 are typical of pastures at the dates nominated, and values for Victorian were calculated from these, based on relative yields of the AVH4 and Victorian.
+ Stocking rate is which can be carried if pasture yield is kept constant (i.e. intake from pasture equals pasture growth rate, assuming low level wastage (20%) of fodder by stock.

5. Future research

It is suggested that two key areas of future research are warranted based on this project. The first area is the on-farm demonstration of the benefits of cultivars such as AVH4 in drought prone areas of temperate Australia that are sown to perennial ryegrass. The second area relates to the use of modern genetic techniques such as molecular marker technologies and genomics to identify genomic regions containing the genes encoding important traits for growth and persistence under water-stressed conditions. Key candidate traits include root growth, fructan biosynthesis and photosynthesis. The adoption of these techniques in breeding programs developing drought tolerant cultivars has the potential to deliver marked increases in the productivity and persistence of perennial ryegrass based pasture in these environments.