LEGUME-RHIZOBIA RELATIONSHIP IN THE NITROGEN FIXATION OF A NEW MEDITERRANEAN PASTURE LEGUME (BISERRULA PELECINUS L.)

A. Loi[1], J.G. Howieson[3], P.S Cocks[2] and S.J. Carr[3]

[1] Co-operative Research Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, Nedlands, WA 6907, Australia
[2] Faculty of Agriculture, The University of Western Australia, Nedlands, WA 6907, Australia
[3] Agriculture, Western Australia, Baron-Hay Court, South Perth, Western Australia 6151

ABSTRACT

Biserrula pelecinus (biserrula) is a pasture legume of Mediterranean climates. Because of its ability to survive on acidic and infertile soils, it has been introduced to southern Australia as a potential alternative or companion plant to serradella and subterranean clover. The successful introduction of this species will be reliant upon the selection of an appropriate inoculant strain of its root-nodule bacteria and understanding of its ecology.

A selection of five rhizobial strains isolated from biserrula nodules collected in Sardinia and Greece were examined for their ability to nodulate and fix nitrogen with 3 genotypes of biserrula.

Although all rhizobial strains nodulated all host genotypes of biserrula, great variability in capacity to fix nitrogen was evident. Distinct PCR amplification profiles were generated for individual rhizobial strains which confirmed the phenotypic variability of the strains. Attention needs to be given to the large host-strain variability for nitrogen fixation in this symbiosis before proceeding with agronomic evaluation.

KEYWORDS

Biserrula pelecinus, Rhizobium, pasture legume, nitrogen fixation

INTRODUCTION

Biserrula pelecinus L. (Biserrula) is a monotypic genus endemic in the Mediterranean areas of Europe and north Africa (Allen and Allen 1981). It has been recently introduced to southern Australia with potential as an alternative pasture legume for acid sandy soils (Howieson *et al.* 1995).

Biserrula attracted our interest because of its frequent occurrence on acid, sandy soils, often as a companion plant to serradella (*Ornithopus compressus*) and subterranean clover (Howieson and Loi 1994). It was often the only legume present on several acidic and infertile sites demonstrating ability to survive in difficult soils (Loi *et al.*, 1995). It has a prosteate growth habit, the pods are easily threshed, and it has small seed with a high level of hardseededness. These attributes suggest it could be a suitable alternative or companion plant to serradella and subterranean clover for acidic sandy soils in the southern Australian agricultural system.

Biserrula has a specific, relatively acid tolerant root-nodule bacteria (Howieson *et al.*, 1995) however, very little is known of genetic variation within either host or rhizobia, or whether rhizobia other than those tested may be effective. The successful adoption of this species by Australian agriculture will be reliant upon the selection of an effective and persistent inoculant strain of its root-nodule bacteria.

The aim of this research was to study the effect of genetic variation in host and rhizobial populations on the effectiveness of the symbiosis between *B. pelecinus* and its microsymbiont.

METHODS

A selection of five rhizobial strains isolated from nodules collected in Sardinia (Loi *et al.*, 1995) and Greece (Carr and Nutt, 1995) were examined for their ability to nodulate and fix nitrogen with three genotypes of *B. pelecinus* (ITA51, MOR99, GRC4) (Table 1). The five strains were also subjected to RAPD analysis with the polymerase chain reaction (PCR).

a) *Nodulation and nitrogen fixation*. Plants were grown in free-draining pots (1 Kg) containing a 50:50 mixture of steam sterilized, nitrogen free coarse river sand and coarse yellow sand covered with alkathene beads. For full details of the nutrient solution composition and microbiological procedures to avoid cross-contamination see Howieson *et al.*, 1995.

The plants and rhizobial material were factorially combined with 3 replications and arranged in randomised blocks. Plants were grown in a naturally lit daylight phytotron (25/20°C day/night) for 7 weeks, the tops were harvested, dried then weighed and root nodulation was assessed by recording the number, size and colour of nodules. Uninoculated and nitrogen fed controls provided the extremes of plant performance for comparison.

b) *PCR amplification*: Total genomic DNA isolated from the five strains was analyzed by the polymerase chain reaction (PCR) in conjunction with a 20-base oligonucleotide primer corresponding to a conserved nif gene promoter region (RPO1) (Richardson *et al.*, 1995).

RESULTS AND DISCUSSION

Although all rhizobial strains nodulated all host genotypes, great variability in capacity to fix nitrogen was found in root-nodule bacteria of biserrula, both within strains and hosts. The yield of the five genotypes of biserrula was greatest with strain WSM1558 and this was very close to the nitrogen control (Fig. 1). Although the strain WSM1566 was collected at the same site as WSM1558, it was moderately effective in nitrogen fixation with genotypes GRC4 and MOR99, while its yield was greater with the Sardinian genotype ITA51.

The low yield of genotypes ITA51 and GRC4 with the strains WSM1456, WSM1575 and WSM 1537, in contrast with the high yield of genotype MOR99 with the same strains caused a significant host-strain interaction ($P<0.05$). In fact, although most of the strains were collected from similar niches in Sardinia, many were only moderately effective in nitrogen fixation with the Sardinian genotype ITA51 ($P<0.05$).

The group of *rhizobium* strains examined in this study was clearly differentiated by PCR amplification using the *nif*-directed primer RPO1 (Fig. 2). In all the cases, distinct amplification profiles were generated for individual strains by RPO1 which confirmed the phenotypic variability of the strains.

Our results show that substantial variability in nitrogen fixation potential exists in root-nodule bacteria which nodulate biserrula. From a breeding perspective, this diversity appears to offer great value for selection of an appropriate inoculant for this species, although attention needs to be given to the large host-strain variability for nitrogen fixation in the symbiosis. Further experiments to investigate the ecology and the persistence of these strains under the

edaphic conditions suiting the host are currently underway. When complete, we will be in a position to recommend the adoption of an appropriate inoculant quality rhizobial strain to industry.

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Table 1
Plant and rhizobial germplasm used in the experiments and some details of the site of origin.

Strains	Host of origin	Country	Characteristics of the site of origin		
			pH	Parent rock	Soil type
WSM1558	Biserrula	Sardinia	6.0	Granitic	Sandy loam
WSM1566	Biserrula	Sardinia	6.0	Granitic	Sandy loam
WSM1537	Biserrula	Sardinia	5.5	Granitic	Sandy loam
WSM1575	Biserrula	Sardinia	7.0	Granitic	Loamy sand
WSM1456	Biserrula	Patmos (Greece)	7.5	Schistic	Clay loam
Genotypes					
ITA51		Sardinia	6.0	Granitic	Loamy sand
GRC4		Serifos (Greece)	6.0	Granitic	Coarse sand
MOR99		Morocco	6.7	Unknown	Sandy loam

Figure 1
The shoot dry weight produced by three genotypes of B. pelecinus when inoculated separately with 5 strains of root-nodule bacteria under controlled conditions (l.s.d., P<0.05 = 0.03)

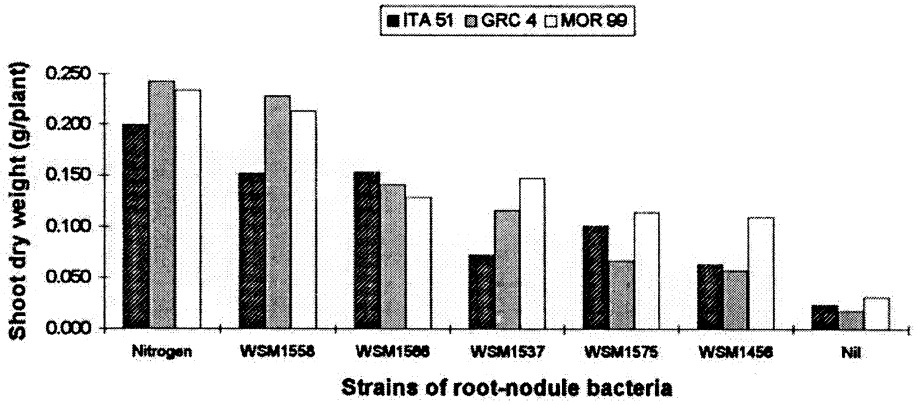

Figure 2
PCR amplification of Rhizobium DNA using a 20-base oligonucleotide primer (RPO1). Lane 1 and 7: Dna marker (SPP-1 Bacteriophage DNA restricted with ECO RI), lanes 2 to 6: strains are in the same order as reported in Fig. 1.