Acid tolerant rhizobia for faba bean grown in south western Victoria

Frank Henry[1], Ross Ballard[2] and Elizabeth Farquharson[2]

[1] Agriculture Victoria, 915 Mt Napier Rd, Hamilton, Victoria, 3300, frank.henry@ecodev.vic.gov.au
[2] South Australian Research and Development Institute (SARDI), Gate 2B Hartley Grove, Urrbrae, SA,5064

Abstract
Poor nodulation of faba beans grown on acid soils (pH_{CaCl2} < 5.5) is considered a major constraint to increasing the area grown to faba beans, increasing grain yield and reducing dependence on artificial fertiliser. To help overcome this problem, rhizobia bacteria with improved tolerance to acidic soils were developed at SARDI and DPIRD. A number of the new acid tolerant strains of rhizobia were compared to the commercial Group F strain WSW-1455 in trials undertaken between 2016 and 2018 within the Victorian high rainfall zone. Several acid tolerant strains showed improved nodulation and grain yield. The rhizobia strain SRDI-970 had significantly higher grain yield (6.2 t/ha) and nodulation score compared to the current commercial strain Group F WSM-1455 (2.2 t/ha) in 2017. While acid tolerant rhizobia are still two years from commercialisation, doubling the rate of the commercial Group F WSM-1455 inoculant may improve nodulation and grain yield providing an interim solution until a new strain becomes available. Acid tolerant rhizobia are not considered a replacement for lime, but rather a complement to a good lime program.

Keywords
Inoculant, bacteria, nodulation, soil acidity, N_2-fixation

Introduction

Increasing the area of pulses grown in the high rainfall zone (HRZ) of south western Victoria is desirable because they provide a disease break option between canola and cereal crops, increase diversity of income and they fix nitrogen (N) which reduces the need for artificial N fertiliser. Faba beans are considered the best pulse option for the HRZ because of their tolerance for wet conditions, compared to other pulses. However, poor nodulation of faba beans grown on acid soils (pH_{CaCl2} < 5.5) is considered by industry as a major constraint to increasing both the area of faba beans grown and their yield potential (Burns et al. 2017).

Faba bean, like all legumes, have a symbiotic relationship with specific rhizobia bacteria that infect their roots and form nodules where they fix nitrogen from the atmosphere. Rhizobia are very sensitive to environmental stresses like pH. The commercial Group F WSM-1455 strain is sensitive to low pH_{CaCl2} (<5.2) which reduces nodulation and N_2-fixation (Drew et al. 2012). However, a promising solution to this problem is the development of acid tolerant strains of rhizobia that will improve nodulation in low pH_{CaCl2} (<5.2) soils (Ballard et al. 2018). The work reported here is part of a broader program evaluating new rhizobia strains for bean and lentil on acid soils.

Methods
Field trials were undertaken in the Victorian HRZ between 2016 and 2018 to compare the rhizobia strain used in commercial Group F faba bean inoculant (WSM-1455) with new acid tolerant (AT) strains of rhizobia developed by South Australian Research and Development Institute (SARDI) and Department of Primary Industries and Regional Development (DPIRD).

Paddocks used for the trials were selected for low pH_{CaCl2} (4.0 - 5.0) and for absence of bean nodulating rhizobia (*Rhizobium leguminosarum* bv. *viciae*), i.e. paddocks without a history of beans, vetch or field pea. Soil samples for background rhizobia were taken using PreDicta B sampling methods. A plant bioassay was also used to assess background soil levels of bean rhizobia. Sites that produced nodulation on field pea roots (nodulated by the same rhizobia as bean) were rejected.

Moist peat inoculants of all rhizobia strains were produced at SARDI and applied to seed as a slurry made by mixing the moist peat culture with 0.5% methyl-cellulose sticker. They were applied to seed one day prior to sowing to maximise the survival rate of rhizobia. The nil control in all the trials also had moist peat, without rhizobia, applied to the seed. In 2016, two fungicide seed treatments Apron XL (350 g/L Metalaxyl-M) and P-Pickel T (360g/L thiram and 200 g/L thiabendazole) were tested because *Pythium* spp and *Rhizoctonia* spp were isolated from areas of poor faba bean growth the year before. Seed was first treated with fungicide and then, in a separate operation inoculated with rhizobia. The inclusion of fungicide seed treatments in the trial allowed the impact of seed treatment on rhizobia survival to be assessed. Plants (6-8 plants/plot) were sampled 8-10 weeks after sowing and scored for nodulation using a 0-5 assessment scale, 0-1 = poor, 2-3 = adequate and 4-5 = good.

Sampling for peak biomass was undertaken at mid pod fill when 10 plants were sampled from trial plots to determine the proportion of legume N derived from the atmosphere by N_2 fixation (%Ndfa) using the ^{15}N natural abundance method (Unkovich et al. 2008). Samples were analysed at the UC Davis Stable Isotope Facility in 2016 and 2018; the WA Biogeochemistry Centre School of Plant Biology in 2017.

Harvest data was recorded as grain yield. Data was analysed using analysis of variance (ANOVA), Fisher's protected least significant difference test and linear regression and in GenStat (18th Edition).

Results and Discussion

Ballyrogan 2016 (pH_{CaCl2} = 4.8)

Soil conditions were optimal for sowing at Ballyrogan on 28 May 2016. For 2016, it was decile 8-9 for rainfall, with slightly above average growing season rainfall associated with a wet September (BoM).

For crop response to treatments, there was a significant (P<0.001, R^2 = 0.55) relationship between nodulation score and grain yield. All the rhizobia treatments produced significantly higher (P = 0.001) nodulation scores and grain yield compared to the nil rhizobia treatment (Table 1). Apron XL seed treatment reduced nodulation and grain yield significantly (P = 0.001) compared to all the rhizobia treatments, while PPT only reduced nodulation and grain yield compared to the best AT strains SRDI-969 and WSM-4643.

The %Ndfa (% legume N derived from atmosphere) was significant higher (P = 0.001) for all rhizobia treatments (average 76%) compared to the nil treatment (26%). However, there was no significant difference between the rhizobia treatments. The best acid tolerant rhizobia treatment SRDI-954 had %Ndfa of 83 and fixed 159 kg/ha of nitrogen (not including root contributions).

Table 1. Effect of fungicide seed treatment and rhizobia strain on nodulation and grain yield of faba bean cv. Samira grown on acid soil (pH_{CaCl2} - 4.8) at Ballyrogan, Victoria, 2016.

Treatment	Nodulation* (0-5)†	Yield* (t/ha)
Nil (no rhizobia)	0.1 a	0.9 a
Group F 1455	2.3c	4.8 cd
[1]Group F 1455 + PPT	2.0 c	3.8 bc
[2]Group F 1455 + Apron XL	0.9 b	3.2 b
AT WSM 4643	1.8 c	5.3 d
AT SRDI 970	2.2 c	4.3 cd
AT SRDI 969	3.0 d	4.8 cd
AT SRDI 954	2.3 c	4.8 cd
LSD (P = 0.05)	0.7	1.0

[1]PPT (P Pickel T, 360g/L thiram and 200 g/L thiabendazole) and [2]Apron XL (350 g/L Metalaxyl-M). †Nodulation assessment based on 0-5 scale: 0-1 = poor, 2-3 = adequate and 4-5 = good. *Values followed by the same letter do not significantly differ according to Fisher's Protected Least Significant Difference (LSD).

In 2016, the rhizobia strains were applied at four times the recommended rate and this may have reduced the difference between the WSM-1455 and new acid tolerant strains of rhizobia. While seed treated with rhizobia and fungicide produced higher nodulation scores and grain yield compared to the nil rhizobia control, Apron XL significantly reduced nodulation and grain yield on all treatments. If a paddock in known to have disease risk, it may be better to use a granule or liquid slurry of the peat inoculant injected into the soil, separating the rhizobia from a toxic fungicide.

Chatsworth 2017 (pH_{CaCl2} = 4.7)

Growing season rainfall was above average at Chatsworth in 2017. However, an extra 20 mm of rainfall during May caused wet conditions at sowing which resulted in poor germination of seed in some plots. September and October were dry with less than half long term-average growing season rainfall (BoM).

For crop response, all the acid tolerant strains of rhizobia produced significantly (*P* = 0.001) higher nodulation scores compared to the nil control, whereas both application rates of commercial inoculant (Group F-1455) produced nodulation similar to the nil (Table 2). Double the recommended rate of commercial Group F WSM-1455 inoculant significantly (*P* = 0.014) increased grain yield by 2.9 t/ha compared to the no rhizobia control. The grain yields of faba bean inoculated with the acid tolerant rhizobia strains, SRDI-969 (5.0 t/ha) and SRDI-970 (6.2 t/ha) were significantly (*P* = 0.014) higher compared to the grain yield of faba beans inoculated with Group F WSM-1455 (2.2 t/ha) (Table 2). On average, this represented a 155% increase in yield due to acid tolerant strains.

While the %Ndfa was higher for all treatments compared to the no rhizobia treatment, there was no significant difference between inoculated treatments. It was a similar story for soil nitrogen fixed by the crop. However, on average %Ndfa for all treatments was 76%, which resulted in 84 kg/ha nitrogen fixed by the crop. While not significantly different, the best acid tolerant rhizobia treatments were SRDI-696 and WSM-4643, which fixed 113 kg/ha and 137 kg/ha of atmospheric N, respectively. These results are similar to Unkovich et al. (2010) who estimated the Australian average %Ndfa for faba beans was 65% and the average crop nitrogen fixed was 96 kg/ha (not including root contributions).

Table 2. Effect of rhizobia strain and inoculant rate on nodulation and grain yield of faba bean cv. Samira grown on acid soil (pH$_{CaCl2}$ 4.7) at Chatsworth, Victoria, 2017. Treatment	Nodulation* (0-5)†	Yield* (t/ha)
Nil (no rhizobia)	0.5 a	1.3 a
Group F 1455 single rate	1.0 ab	2.2 ab
Group F 1455 double rate	1.2 ab	4.2bcd
AT WSM 4643	2.2 cd	2.9abc
AT SRDI 971	1.5bcd	4.5bcd
AT SRDI 970	2.3 d	6.2 d
AT SRDI 969	1.4 bc	5.0 cd
AT SRDI 954	1.5 bc	3.2abc
LSD (*P* = 0.05)	0.8	2.3

†Nodulation assessment based on 0-5 scale: 0-1 = poor, 2-3 = adequate and 4-5 = good.*Values followed by the same letter do not significantly differ according to Fisher's Protected Least Significant Difference (LSD).

Stawell 2018 (pH$_{CaCl2}$ = 4.7)

At Stawell in 2018, dry seasonal conditions (decile 1) prevailed with September only receiving 15% of its growing season rainfall (BoM). These conditions severely affected plant growth and grain yield.

Table 3. Effect of rhizobia strain and inoculant rate on nodulation and grain yield of faba bean cv. Samira grown on acid soil (pH$_{CaCl2}$ - 4.2) at Stawell, Victoria, 2018.

Treatment	Nodulation* (0-5)†	Yield* (t/ha)
Nil (no rhizobia)	0.3 a	0.21 a
Group F 1455 single rate	1.1 b	0.97 c
Group F 1455 double rate	1.5 c	0.76 bc
AT SRDI 969 single rate	0.9 b	0.72 bc
AT SRDI 954 double rate	1.2 bc	0.83 bc
AT WSM 4643	0.9 b	0.74 bc
AT SRDI 970	0.9 b	0.56 b
AT SRDI 954	0.9 b	0.65 bc
AT SRDI 1000	1.0 b	0.72 bc
LSD (*P* = 0.05)	0.4	0.3

†Nodulation assessment based on 0-5 scale: 0-1 = poor, 2-3 = adequate and 4-5 = good.*Values followed by the same letter do not significantly differ according to Fisher's Protected Least Significant Difference (LSD).

Although nodulation scores were low (≤ 1.5), all rhizobia treatments improved nodulation significantly (*P* = 0.001) compared to the nil rhizobia treatment. It is likely that low soil pH $_{CaCl2}$ 4.2 and not dry conditions at this site, limited the nodulation, as plants were assessed for nodulation in August, when conditions were still favourable, before the very dry September. Doubling the rate of SRDI-954 and Group F WSM-1455 inoculant produced the highest nodulation scores of 1.2 and 1.5 respectively (Table 3).

In this trial there was a poor relationship (R^2 = 0.41) between inoculation score and grain yield, most likely due to the low soil pH_{CaCl2} 4.2. All the seed treated with rhizobia produced significantly (*P* = 0.001) higher grain yields, compared to the nil rhizobia control, but grain yield was considerably lower than in other experimental years due to dry spring conditions and possibly the lower site pH. Seed treated with Group F WSM-1455 inoculant at the single rate produced the highest grain yield of 0.97 t/ha, but was not significantly different to other treatments except for SRDI-970.

Conclusion

New acid tolerant strains of rhizobia show potential to increase faba bean nodulation and grain yield on acid soils, where the lower limit of their benefit is around pH_{CaCl2} 4.2. New rhizobia options are two years from commercial production. In the short term, using a double rate of Group F WSM-1455 inoculant may provide a practical way to increase nodulation and yield potential in faba bean on acid soils. This approach resulted in an improvement in nodulation at Stawell, a trend for increased grain yield at Chatsworth and is thought to have supported better nodulation at Ballyrogan where all strains were applied at four times the commercial rate. It has without doubt improved nodulation in other trials on acidic soils (Ballard et al. 2018) and is linked to an increased number of rhizobia surviving at the time of nodulation.

When improved acid tolerant rhizobia become available they should not be considered a replacement for liming. Liming to about pH_{CaCl2} 5.0 provides broader benefits to plant growth and will remain an important practice where soil pH is below the level where the acid tolerant rhizobia provide a benefit (e.g. at Stawell). Lime should be applied at least one season ahead of planting faba bean to allow enough time for the lime to dissolve into the root zone.

Overall, this work will provide the industry with compelling information that demonstrates how acid tolerant rhizobia combined with a robust liming program can increase yield and the area suitable to faba bean production on acid soils in south western Victoria.

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