Enhancing the economic potential of beans using *Vicia faba* L.: Crop performance and the use of air-classified grain components in animal feeds and brewing

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Abstract: Research at the James Hutton Institute's Centre for Sustainable Cropping (CSC) shows biological nitrogen fixation by faba beans (*Vicia faba* L.) is maximal under low input, non-inversion tillage with compost incorporated. Increasing bean protein production efficiency is being investigated with respect to rhizobial diversity and through development of the supply chain. Powdered faba bean kernels are fractionated into bean-protein and -starch concentrates (BPC and BSC, respectively) using 'air-classification'. BPC can displace soya and fishmeal in salmon-feed without impacting fish growth, feed efficiency or health. BSC performed well in poultry- and pig-feeds and has significant socio-economic potential in brewing.

Key words: aquaculture, distilling, nitrogen-fixation, protein, rhizobia

Introduction

The socioeconomic potential of whole legume grains maybe greatest where yields are high, stable and grains possess the highest possible protein content. In addition, fractionating the grain can increase economic returns by diversifying the range of legume-based products which are available. Any consequent increase in gross margins would encourage greater uptake of legume supported cropped systems and so enhance sustainable production. Towards that end, we report on: 1) the performance of faba beans (*Vicia faba* L.) with respect to nitrogen fixation and rhizobial diversity under conventional and sustainable agronomic treatments; 2) the efficacy of "air classification", a low cost method which uses a vertical cyclonic air stream to generate bean protein- and bean starch-concentrates (BPC and BSC, respectively; Fig. 1) from milled faba beans (kernels), and 3) the utility of the BPC and BSC in animal production and brewing.

Materials and methods

Faba bean yield, the proportion of nitrogen (N) derived from air (%Ndfa), biological nitrogen fixation (BNF; 4), and molecular diversity of nodule-associated *Rhizobium leguminosarum* (1, 3, 5, 6) of five bean varieties were quantified in response to 'conventional' and 'sustainable' growing regimes at the Centre for Sustainable Cropping (CSC; www.hutton.ac.uk/csc), over three growing seasons (2011-2013). The standardised ileal digestibility (SID) of BSC essential amino acids (AA) was determined in 28 d old birds and 70 d old pigs. Fish growth, feed efficiency and intestinal inflammation were monitored in freshwater Atlantic salmon parr (initial body weight 1.5 g, 18 feeds tested for 56 d) and sea water Atlantic post-smolt salmon (initial body weight 1.276 g, nine feeds tested for 112 d). The potential of whole faba beans (WFB) and BSC was assessed as an adjunct using serial combinations with malted barley grist (WBG) in ale brewing trials at laboratory- and pilot-brewery-scale.

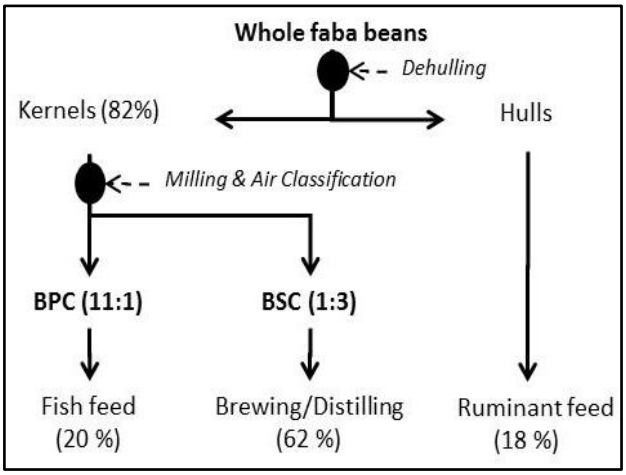

Figure 1. A schematic diagram showing the possible fates of whole faba bean components: including the hulls and bean protein concentrates (BPC) and bean starch concentrate (BSC), derived from the air classified kernels; percentages relate to proportions of whole bean; ratios show the proportion of protein : starch

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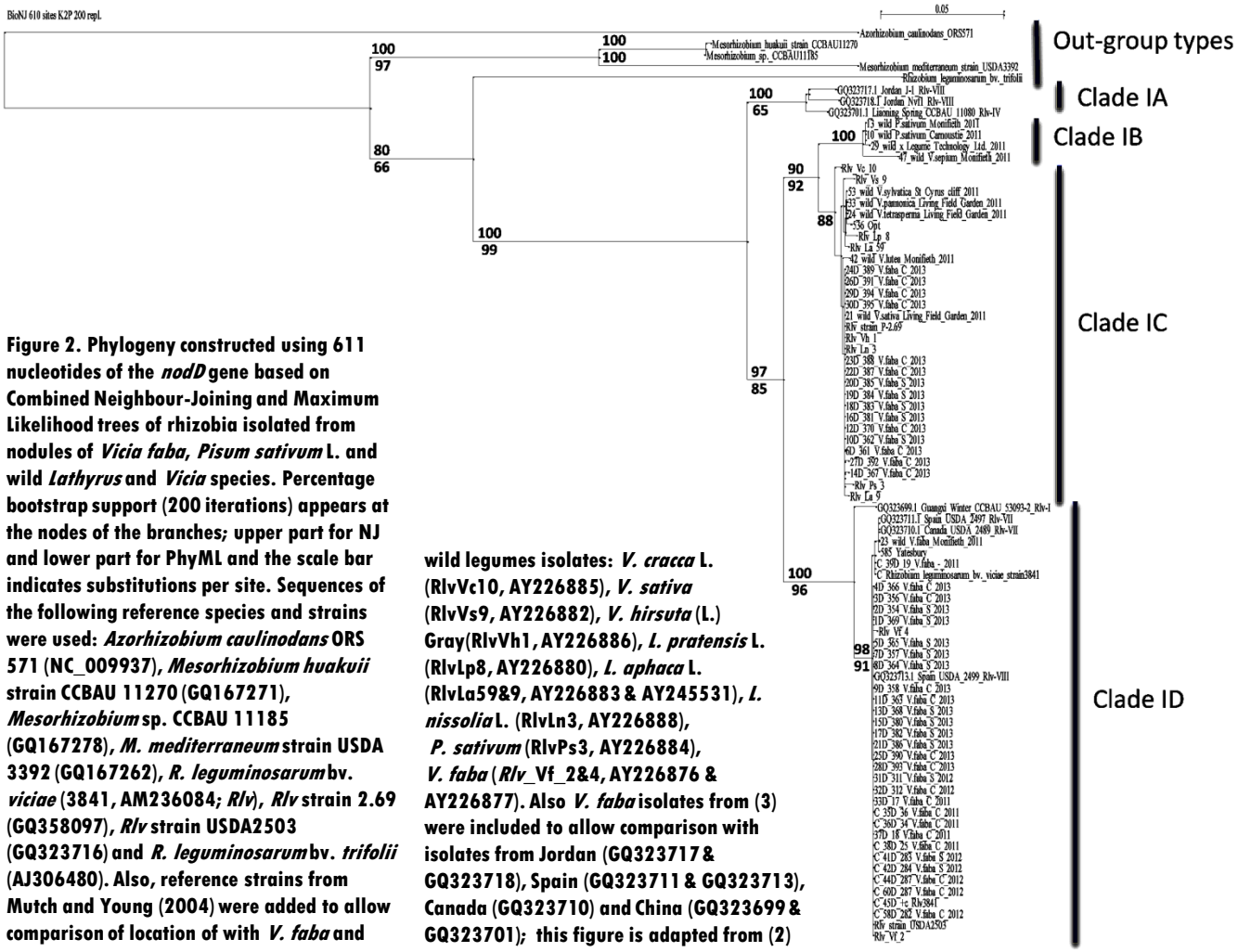

Figure 2. Phylogeny constructed using 611 nucleotides of the *nodD* gene based on Combined Neighbour-Joining and Maximum Likelihood trees of rhizobia isolated from nodules of *Vicia faba*, *Pisum sativum* L. and wild *Lathyrus* and *Vicia* species. Percentage bootstrap support (200 iterations) appears at the nodes of the branches; upper part for NJ and lower part for PhyML and the scale bar indicates substitutions per site. Sequences of the following reference species and strains were used: *Azorhizobium caulinodans* ORS 571 (NC_009937), *Mesorhizobium huakuii* strain CCBAU 11270 (GQ167271), *Mesorhizobium* sp. CCBAU 11185 (GQ167278), *M. mediterraneum* strain USDA 3392 (GQ167262), *R. leguminosarum* bv. *viciae* (3841, AM236084; *Rlv*), *Rlv* strain 2.69 (GQ358097), *Rlv* strain USDA2503 (GQ323716) and *R. leguminosarum* bv. *trifolii* (AJ306480). Also, reference strains from Mutch and Young (2004) were added to allow comparison of location of with *V. faba* and wild legumes isolates: *V. cracca* L. (RlvVc10, AY226885), *V. sativa* (RlvVs9, AY226882), *V. hirsuta* (L.) Gray(RlvVh1, AY226886), *L. pratensis* L. (RlvLp8, AY226880), *L. aphaca* L. (RlvLa59&9, AY226883 & AY245531), *L. nissolia* L. (RlvLn3, AY226888), *P. sativum* (RlvPs3, AY226884), *V. faba* (*Rlv*_Vf_2&4, AY226876 & AY226877). Also *V. faba* isolates from (3) were included to allow comparison with isolates from Jordan (GQ323717 & GQ323718), Spain (GQ323711 & GQ323713), Canada (GQ323710) and China (GQ323699 & GQ323701); this figure is adapted from (2)

Results and discussion

Field data indicated that %Ndfa was highest under sustainable management, with mean %Ndfa values (and %Ndfa increase relative to conventional management) across five cultivars of 87% (+11%) in 2011, 91% (+8%) in 2012 and 87% (+6%) in 2013. BNF was also higher under sustainable treatment with an average of 25 kg ha^{-1} more N remaining in the soil compared to conventional practice. Across both treatments, BNF ranged from 150 kg N ha^{-1} - 300 kg N ha^{-1} with 70% - 90% of the fixed N removed in grain and 25 kg ha^{-1} - 80 kg ha^{-1} remaining in-field.

Rhizobial diversity studies characterised isolates as distinct from the 'out-group' bacterial types (*Mesorhizobium* and *Azorhizobium*; Fig. 2), and from those isolates known not to nodulate this crop at their diversity centre ('Clade IA'). Thus, the phylogenetic tree showed that rhizobia isolated from faba bean cultivated at the CSC co-localised either with rhizobial types isolated from wild legumes (Clades IB and IC) or *Rlv* (Clade ID). Comparative functional characterisation of the isolates belonging to distinct clades is ongoing.

Salmon feeding studies showed that fish growth was faster or feed conversion more efficient when BPC was included in the feed. Intestinal health of fish was not compromised at levels below 30% inclusion of BPC, indicating the utility of faba bean BPC in salmon feeds.

Pigs and poultry feeding trials showed that the SID of BSC essential AA averaged 84.8 ± 1.6 % and 83.3 ± 2.0 % in pigs and poultry, respectively. Provided its relatively low methionine content is supplemented BSC may be an attractive alternative protein source to reduce reliance on soya bean meal for pigs and poultry.

Brewing trials tested different fermentation methods and enzymatic combinations and identified a novel zymology-based method to produce an ale of 5.0% ABV with good sensory characteristics using 25% [w/w] BSC.

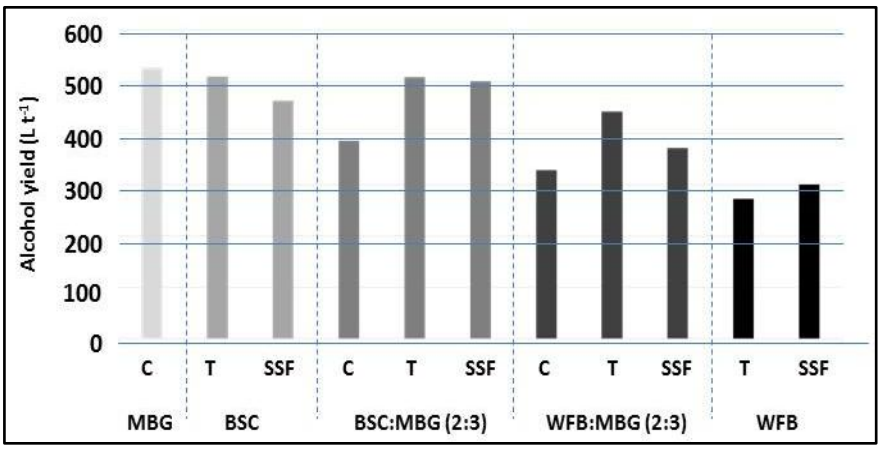

Figure 3. Alcohol yields of bean starch concentrate (BSC), malted barley grist (MBG) and whole faba bean (WFB) flours alone, or in combination, using three different fermentation systems: conventional (C), using the natural diastatic power of the MBG; traditional (T), using added α-amylase and glucoamylase in the 'mash', or simultaneous saccharification and fermentation (SSF), using α-amylase in the mash and added glucoamylase when fermentation is initiatedl; ratios relate to the respective percentages of wort adjuncts

The BSC and milled WFB (kernels) can be used alone or in combination with MBG to achieve good alcohol yields (Fig. 3). The three saccharification methods trialed were: conventional (C), employing the natural diastatic power of the MBG; traditional (T), using added α-amylase and glucoamylase in the 'mash', or; simultaneous saccharification fermentation (SSF), using α-amylase in the mash and added glucoamylase when fermentation is initiated -to facilitate the slow and continual release of glucose throughout fermentation. The alcohol yields were highest and similar to MBG-alone when the T and SSF methods were applied to BSC:MBG, or using the T method on BSC-only. Current work aims to identify methods which optimise alcohol production using the BSC and WFB-kernelflours and help develop legume-grain-only (*i.e.* gluten-free) products. See (7), for a full report of this brewing and distilling research: or contact the author.

Acknowledgements

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