The physiology and biochemistry of cultivar-strain interactions in the white clover-Rhizobium symbiosis

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Abstract

Two white clover cultivars were inoculated with two Rhizobium leguminosarum bv. trifolii strains in a factorial series of experiments. Plants were grown in axenic conditions in nitrogen free nutrient solution in a controlled environment room. Variations in nitrogen fixation were dependent partly upon general strain effects, partly upon general cultivar effects but there were also substantial differences attributable to precise interactions between specific combinations. The physiological and biochemical basis of these differences was examined. There were variations in the onset of nodulation and nitrogenase (acetylene reduction) activity. The rate at which nitrogenase activity developed also differed between associations as did the average size and number of nodules but none of these effects correlated well with differences in plant dry matter accumulation. Studies on nodule biochemistry revealed that the major nitrogen fixation enzymes were present in all four associations. Nodule protein content and enzyme activity (on a g nodule fresh weight basis) were substantially greater in associations formed by the more effective strain but cannot explain the interactive effect on dry matter accumulation. The relevance of these data to our understanding of factors regulating variations in nitrogen fixation is discussed.

Introduction

The productivity, in terms of dry matter and nitrogen accumulation, of white clover/Rhizobium associations is determined by the effectiveness of the interaction between the symbionts. While some cultivar and strain characteristics show general trends over a range of symbiotic pairs, such as one strain being particularly effective in comparison with another, these trends are not completely predictable. One particular rhizobial strain does not always outperform another over a range of cultivars, neither is one cultivar necessarily superior to another over a range of strains (Mytton, 1975). Similar interactive effects have been found in faba beans (Mytton, et al., 1977) and peas (Cresswell, 1990). At present, compatibility screening of cultivars and strains is necessary to evaluate this synergistic effect. Understanding of the basis of the interactive effect will obviate the need for screening and may highlight crucial elements of the nitrogen fixation process which are contrasted in these associations.

Screening trials conducted in 1986 at the Welsh Plant Breeding Station on 30 Rhizobium strains isolated from the field identified two cultivars, Katrina (K) and S100 (S), and two strains, JRN1/4 (J) and LH15/1 (L) which showed clear interactions; strain L was more effective with K than with S while J was more effective with S than with K.

Investigation of the basis of the interaction was split into three areas. Firstly, the speed with
which an association initiates nitrogen fixation, since this could have a cumulative developmental effect. Secondly, the efficiency of use of photosynthate in nitrogen fixation, since an association which uses relatively small amounts of carbon per N₂ molecule fixed could be at an advantage over one with higher carbon costs, (Skot et al., 1986). Thirdly, the size of the nitrogen fixing apparatus in relation to the size of the plant, since a greater investment in nodules and in the proteins related to nitrogen fixation could lead to greater plant productivity in the longer term.

Materials and methods

General

Tube culture of clover plants
White clover seedlings of cultivars Katrina (K) and S100 (S) were grown in axenic conditions in 60 cm³ test tubes (Rys and Mytton, 1986). Briefly, a 15 mm x 150 mm filter paper wick was folded in half and placed in a tube to provide support for a seedling. 20 cm³ of Jensons N-free nutrient solution was added to each tube which was then capped with a loose fitting lid. The tube, cap, filter paper and nutrient solution were autoclaved before a 1-day-old clover seedling, germinated on water agar in sterile conditions, was transferred onto the filter paper. Tubes were then inoculated with the appropriate strain, LH15/1 (L) or JRN1/4 (J) before being moved into a controlled environment (day/night regime 16/8 hours at approximately 600 µmol m⁻² s⁻¹ and 20/15°C).

Plant material analysis
Shoot, roots and nodules were dried for 24-48 hrs at 85°C before weighing. The fresh volume of nodules on a plant, or set of replicate plants, were obtained by displacement of water in a 1-cm³ syringe. Total nitrogen content and % N content of shoots, roots and nodules were determined using a Carlo Erba nitrogen analyser.

Experiments 1 and 2
Experiment 1 assesses the pattern of growth and development of nitrogen fixation capacity over the first seven weeks of seedling growth in order to reveal the age at which the interaction first becomes observable. Experiment 2 investigates the length of the lag between inoculation of the seedling and the onset of fixation.

Clover plants were grown in tubes for a total of seven weeks. Sequential harvests were taken at progressively longer intervals during this time. Acetylene reduction activity (ARA) was measured in a closed system to determine the time of onset and initial rate of fixation. Due to small plant size (<100 mg fresh weight per plant before week five) detection of ARA depended on bulking replicate plants. For each treatment at each harvest, 10 seedlings were placed on wet filter paper in one 60 cm³ tube, sealed with a subaseal and 10% of the air removed by syringe and replaced with acetylene. Gas samples of 0.5 cm³ were withdrawn from the tube at intervals for ethylene quantification by gas chromatography. No more than 5% of the total volume of a tube was taken in samples. Nodule numbers, shoot dry weight, and root (including nodules) dry weight were recorded at each harvest.

Harvest intervals at the beginning of Experiment 1 were too long to determine the onset of fixation for all the four associations. In Experiment 2 harvests were taken at daily intervals until all treatments had commenced fixation.

Experiment 3: Carbon costs and nodule parameters
Clover plants were grown in tubes for assessment of the energy cost of reduction of acetylene, which can be related to the carbon costs of nitrogen fixation (Witty et al., 1983). This necessitates use of a flow-through acetylene reduction system. In experiment 3 plants were harvested at 22, 31, 42 and 49 days after inoculation. Ten replicate plants at each harvest were bulked together to ensure detection of activity and placed in a blackened glass vessel attached in line with a gas flow-through system connected to an infra red gas analyser for CO₂ assessment, a H₂ detector (Layzell et al., 1984) and a gas chromatograph for C₂H₄ quantification.

At the end of seven weeks, a sample plant from each treatment was potted on from the tubes into sterile vermiculite, reinoculated and