

CULTIVAR VARIATION IN THE NODULATION OF WHITE CLOVER SEEDLINGS PROVIDED WITH NITROGEN

G.J. Rys

Agricultural Research Division
Ministry of Agriculture & Fisheries
Hastings, New Zealand

ABSTRACT

More than 3800 white clover seedlings from 30 cultivars were screened for their ability to nodulate when provided with a high NH_4NO_3 level. Nodulation was assessed in seedlings growing in nutrient solution under sterile controlled environment conditions. Eleven cultivars had less than 10% of plants nodulated. Two cultivars, Karina and an irradiated line of Huia, had more than 60% of plants nodulated. Only one cultivar, Tamar, had more than three nodules per nodulated plant.

Intra cultivar variation of cultivar S.100 provided with a high NO_3^- level was evaluated using the same technique. From 110 seedlings, five were selected for high (H), low (L), and zero (0) nodules. The five plants in each group were polycrossed. Progeny from one polycross parent of each group were evaluated for ability to nodulate in abundant NO_3^- . Nodule numbers varied from zero to 20 in the unselected population, with the mean nodule numbers of the five selected plants in each group being 13.0 (H), 1.2 (L), and 0 (0). The nodule formation rate of progeny from the H nodule parent was 0.172 nodules/plant per day compared to 0.060 and 0.058 for the L and 0 progeny.

Considerable variation existed both between and within cultivars for the ability to nodulate in the presence of combined nitrogen (N). The ability to nodulate under high N had a heritable component, and does not appear to be related to nodulation in the absence of N.

KEYWORDS

Trifolium repens L., *Rhizobium leguminosarum* biovar *trifolii*, NO_3^- .

INTRODUCTION

White clover (*Trifolium repens* L.) through the symbiotic association with *Rhizobium leguminosarum* (Frank) biovar *trifolii* is the largest contributor of N to pastoral agriculture in New Zealand (Steel, 1982). Despite this, N still remains a major nutrient limitation to plant growth. Attempts to overcome this limitation by increasing symbiotic N inputs of white clover may be unsuccessful

because high soil N levels reduce symbiotic activity (Allos and Bartholomew, 1955). The solution may lie in identifying white clover-rhizobia associations that are less sensitive to high soil N levels.

In white clover seedlings, the inhibition of N fixation by combined N occurred through a reduction in nodule numbers rather than in the size of individual nodules, and by reduced N fixation per unit of nodule mass (Rys, 1982). Therefore, the first stage in obtaining a N-tolerant symbiosis is to maximise the plants' ability to form nodules in the presence of high levels of combined N.

Several researchers have modified white clover nodule characteristics, including number and size, by host plant selection in the absence of N (Jones and Burrows, 1968; Connolly *et al.*, 1969). Little information is available on the variation and degree to which selection might modify nodule characteristics in the presence of combined N.

MATERIALS AND METHODS

White clover seedlings were grown in aseptic conditions in nutrient solutions contained within test tubes. Seed was scarified, surface sterilised, and allowed to imbibe water for eight hours. Seed was then germinated on water agar at 25°C, for 24 hours before sowing.

Inoculation was carried out by placing 4 ml of a *Rhizobium leguminosarum* biovar *trifolii* suspension containing 10^7 to 10^8 cells/ml onto germinated seeds in the agar plates before sowing. The rhizobium had been grown in yeast mannitol broth using standard techniques (Vincent, 1970).

Plants were grown in a controlled temperature room at a constant 20°C and a 16-hour photoperiod at a light intensity of 40-45 W.m^{-2} in the inter cultivar comparison and 60-65 W.m^{-2} in the intra cultivar trial.

Inter cultivar variation

Thirty white clover cultivars of varying leaf size from numerous geographical locations were supplied by the Grasslands Division, DSIR, Palmerston North. Between 80 and 300 seedlings per cultivar were evaluated. Each cultivar was evaluated in a separate experiment. Two seeds were sown into each tube containing 3.75 mg of N as NH_4NO_3 .

This level was in excess of that required for growth of both plants (Rys, 1982). Seed was inoculated with a single effective strain (PDD 2668). Plants were grown for 26 to 32 days and then nodules were counted and plant wet weight determined. Ten to 20 seedlings of each cultivar were also grown without N.

Intra cultivar variation

Cultivar S.100 was chosen to evaluate intra cultivar variation. One hundred and ten seeds were sown individually into test tubes containing 8 mg of N as NaNO_3 . Plants were inoculated with a 10 strain mixture (Welsh Plant Breeding Station collection). At the conclusion of the experiment (Day 27), measurements on nodulation and growth parameters were taken. Nodule mass was estimated using the method of Mytton (1973). Acetylene reduction was assayed using standard techniques (Rys and Phung, 1984).

From this trial, plants were selected on the basis of contrasting nodule number and placed into high (H), low (L), and zero (0) nodule number categories. Five similar plants were chosen to form each group. Each group was isolated and seed was obtained after cross pollination using bees. Progeny from one of the five polycross parents in each group was tested against the original population.

Fifty progeny from the selected parent and 50 plants from the original population were grown in test tubes containing 4 mg of N as NaNO_3 . Nodule numbers were determined at regular intervals. The experiment was laid out in a randomised block design. Further details are provided by Mytton and Rys (1985).

RESULTS AND DISCUSSION

Inter cultivar variation

The mean number of nodules formed on nodulated plants was 1.7. An average of only 24% of plants formed nodules. The highest nodule number found on an individual plant was 15.

The distribution of the percentage of plants nodulated within each cultivar for all 30 cultivars is presented in Fig. 1(a). A distinctly skewed distribution is evident with 37% of cultivars having 10% or less plants forming nodules. Two cultivars, Karina and a Huia seed lot which had been irradiated with Co^{60} receiving a dose of 15 kilorads, had the highest proportion of plants nodulated, 62% and 63% respectively. The distribution of the mean nodule number of nodulated plants within each cultivar for all 30 cultivars is presented in Fig. 1(b). Only one cultivar, Tamar, had more than three nodules per nodulated plant. In general the greater the proportion of plants nodulated the greater the mean nodule number of nodulated plants ($r=0.53$, $P<0.01$). However, there were exceptions such as Tamar which had the highest mean nodule number but only 5% of plants nodulated. No significant correlation ($r=0.14$) was found between the nodule number on plants nodulated in the presence of N and the nodule number of plants of the same cultivar grown in the absence of N. This suggests that

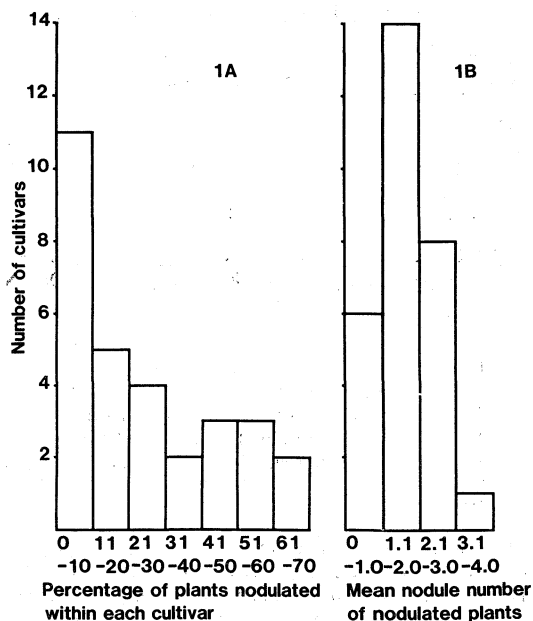


Figure 1. (a) The distribution of the percentage of plants nodulated within each cultivar. (b) The distribution of mean nodule number of nodulated plants within each cultivar, for 30 cultivars of white clover.

any tolerance of plant nodulation to combined N is not related to nodulation ability in the absence of N.

No correlation was found in this experiment between plant wet weight and either the proportion of plants nodulated ($r=0.07$) or the mean nodule number of nodulated plants ($r=0.14$).

Intra cultivar variation

Table 1 shows the considerable variation in characters measured. By Day 27, nodule numbers varied from 0 to 20 per plant. Plants selected for high (H) nodule number averaged three times more nodules than the mean of the unselected population, and more than three times the nodule mass but only 18% higher plant wet weight. The low (L) nodule selections had four times fewer nodules whilst wet weight was 13% greater than the unselected population. The 0 selections had similar wet weight to the mean of the original population.

Nodule numbers closely reflected plant total nodule dry matter ($r=0.79$, $P<0.001$, Table 2). Plant wet weight was not correlated with nodule number, mass or acetylene reduction activity. Acetylene reduction activity was significantly ($P<0.001$) but poorly correlated with nodule number ($r=0.37$), total nodule mass ($r=0.43$), and individual nodule mass ($r=0.41$).

Table 1. Mean and range of growth and symbiotic traits in a population of *Trifolium repens* (cv. S.100) when inoculated and grown with abundant $\text{NO}_3^- \text{N}$ and means of five high, low, and non-nodulating plants.

Selection group	Wet weight (mg/plant)	Nodule number (per plant)	Est. total nodule DM (mg/plant)	Individual nodule DM (mg/nodule)
Mean	473	4.3	0.057	0.012
Range	154-819	0-20	0-0.284	0-0.048
High nodule no.	560	13.0	0.185	0.014
Low nodule no.	536	1.2	0.010	0.009
Zero nodule no.	472	0	0	0

Table 2. Correlation coefficients between yield and nodulation parameters of an unselected *Trifolium repens* (cv. S.100) population provided with a high level of $\text{NO}_3^- \text{N}$ (n = 110).

Variety No.		1	2	3	4	5
1	Plant wet weight (mg/plant)	—				
2	Nodule number per plant (Day 27)	0.14	—			
3	Estimated total nodule DM (mg/plant)	0.13	0.79 ¹	—		
4	Estimated individual nodule DM (mg/nodule)	0.16	0.18	0.59 ¹	—	
5	Acetylene reduction (nmol/plant per hour)	0.05	0.37 ¹	0.43 ¹	0.41 ¹	—

¹P < 0.001

The pattern of nodule formation of progeny from the H, L., and 0 nodule selection parent is presented in Fig. 2. Significant differences did not emerge until Day 10. By then the populations fell into two distinct groups. The H selection line together with the control population averaged 1.6 nodules per plant whilst the L and 0 lines averaged 0.3 nodules per plant.

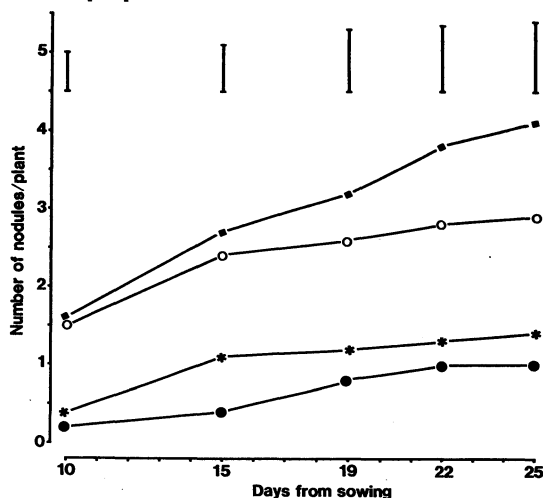


Figure 2. Nodulation in abundant $\text{NO}_3^- \text{N}$ of progeny from plants selected for high, low, or zero nodulation when provided with abundant $\text{NO}_3^- \text{N}$.

* high nodule number, □ low nodule number, ● zero nodule number, ○ control. Bars represent HSD (P = 0.05).

The nodule formation rates were essentially linear over the following 15 days averaging 0.093, 0.172, 0.060, and 0.058 nodules per plant per day for the control, H, L, and 0 lines respectively.

Variation in nodule number between the 50 progeny within each selection line was large with ranges of 1-11, 0-5, and 0-7 for the H, L, and 0 lines respectively. Fifty six percent of plants in the 0 line were without nodules compared with 22% in the L line. All plants in the H line had nodules. Nodule numbers in the control population ranged from 0 to 7 per plant with only 4% of plants un-nodulated.

The mean nodule number in the S.100 population was higher than that in the cultivar comparison trial. This can be attributed to the use of NH_4NO_3 rather than NaNO_3 in the cultivar comparison. NH_4NO_3 has been shown to be more inhibitory on nodulation in white clover than $\text{NO}_3^- \text{N}$ alone (Rys and Phung, 1984). Considerable variation exists both between and within white clover populations in their ability to nodulate in the presence of combined N. Selection and hybridisation can bring about genetically-determined shifts in nodulation ability in the presence of N. Increases in nodule number were not as easy to achieve as reductions. However, considerable variation still existed suggesting advances could be made through further cycles of selection. The number of nodules formed on nodulated plants provided with N was only 25% of the nodule numbers formed on plants growing in the absence of combined N.

The magnitude of the increase required suggests this may be difficult using traditional plant breeding techniques. However, the recent identification of super nodulating plants of soybean (Carroll *et al.*, 1985) and peas (Jacobsen and Feenstra, 1984) using induced mutations, which still develop high nodule numbers in the presence of combined N and maintain N fixation, suggests that it may

be possible to make more rapid advances by obtaining similar mutants in white clover.

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