A GRASSLANDS DIVISION PROGRAMME TO BREED A WHITE CLOVER WITH IMPROVED PHOSPHATE NUTRITION.

J. Dunlop, G.S. Wewala*, J.R. Caradus, A.D. Mackay, M.G. Lambert, A.L. Hart, J. Van den Bosch, M.C.H. Mouat and M.J.M. Hay

Grasslands Division & Applied Mathematics Division, DSIR, Private Bag, Palmerston North.

ABSTRACT

A cultivar of white clover which would permit lower inputs of phosphatic fertilisers without decreasing production would be valuable to pastoral farmers. Grasslands Division, DSIR, has this as a principal goal for its work in mineral nutrition. To proceed from the extensive background research to cultivar we have devised a programme which will move through several phases. The phases include screening, heritability testing, field testing, characterisation of the physiology and morphology of promising material and, eventually, breeding.

This programme involves a number of assumptions. These include the usefulness of pot trials for predicting agronomic performance, that there are differences in phosphate nutrition that are heritable, and that phosphate response curves will provide an efficient path to the desired material.

The initial phase has examined the phosphate response of 120 cultivars of white clover. This indicated wide variation in phosphate nutrition among cultivars.

INTRODUCTION

A phosphate efficient white clover plant has been a gleam in the eye of scientists at Grasslands Division for some time. The first publication motivated by this goal was the paper by Mouat (1962) on genetic variation in root cation exchange capacity. Jackman and Mouat together then conducted several studies (Jackman and Mouat, 1970; 1972a,b).

These ideas were being mulled over at a time when phosphate costs were significant but farming was prosperous and New Zealand's position on the British Phosphate Commission assured us of an inexpensive source of high quality phosphate rock. Consequently they were not given a high priority. They were not unique to New Zealanders. Epstein (1972) wrote of the need to develop plants whose mineral nutrition was suited to the soils in which they were grown rather than changing the nutrient status of soils to suit the plants. However, this has not been a simple goal to achieve and we are unaware of any cultivars whose mineral nutrition has been intentionally improved through breeding.

Over the past decade Grasslands Division staff have investigated several lines of research related to genetically improving the phosphate nutrition of white clover. There have been several attempts to select phosphate efficient plants (Caradus and Dunlop, 1978; Caradus, 1979). These included selection on the basis of growth and also for specific physiological characters considered to be of possible value. Variation was found but there were also large interactions with the environment which made extrapolation from the environment of selection to the field impractical (Caradus, 1985). This work has indicated a need for more information on the factors which determine the growth of white clover in the field.

This need has been in part met by extensive studies on the absorption and metabolism of phosphate by white clover plants. Some of these have been reviewed in this symposium by Dunlop (1988) and Hart (1988). Others are reported by Caradus (1983) and Caradus and Snaydon (1987 a,b,c). This information has given valuable insights into the processes which are significant to the growth of plants. They suggest a number of possible screening parameters. However, it has been difficult to obtain data on the relative quantitative contribution each process makes to growth in the field and we are not yet confident that we can predict agronomic performance from parameters measured in laboratory or glasshouse studes.

Ecophysiological research into white clover growth in pastures at Grasslands Division is motivated partly by the need for information on how to extrapolate from glasshouse and laboratory date to predict performance in the field. A number of scientists have worked in this area and their work is described in Chapman, (1983, 1986); Chapman *et al.,* (1984) and Hay *et al.,* (1986, 1987). Although this information is available to our planning, all ideas on strategies to breed plants with improved mineral nutrition are theorising until a plant is produced. Progress would be accelerated and we would be more confident of our direction if we had material with repeatable differences in its phosphate nutrition. It would enable us to test our concepts and reduce the contingencies that we have to allow for. Furthermore the identification of such material would be very encouraging as it may provide the basis for a breeding programme. For these reasons finding white clover plants with consistent differences in phosphate nutrition has become an important intermediate goal in our work.

Our work so far convinces us that breeding a white clover plant with improved phosphate nutrition is a complex task that requires a broad range of expertise. Furthermore much of the work is labour intensive, requiring large coordinated efforts. The current economic pressures on pastoral farming, recent steep rises in the costs of applying fertiliser and potential benefits of an easing of these burdens by breeding a suitable cultivar of white clover have provided the justification and impetus to form a group with the resources needed.

This paper outlines and summarises progress and the work planned.

BASIC CONCEPTS

The fundamental premise of this programme is that in white clover — grass swards the clover dictates the high requirement for phosphate. This is based on the work of Jackman and Mouat (1972a,b) who found that white clover competed ineffectively with grasses for scarce phosphate. It is considered that if the performance of white clover at low phosphate can be improved sward productivity could be maintained with less phosphate. Pasture productivity has been shown to be closely related to legume performance in that the low level of available soil nitrogen is the primary nutritional limitation and stimulation of the legume — rhizobium symbiosis alleviates nitrogen deficiency (Lambert *et al.,* 1983, 1986; Lambert, 1987).

We are proceeding as if performance measured in glasshouse pot experiments will provide information that is relevant to the field even though the extrapolation is not simple and previous attempts have not succeeded. However, this course is forced upon us. Screening in the field is not practical because of the mosaic of fertility and other conditions affecting growth in all potential sites. We consider that it will be essential to test conclusions based on data from pot trials against results from the field at the earliest possible stage.

We have decided to initially categorise plants on the basis of the shape of their phosphate response curves rather than performance at a single level of phosphate. There are several reasons for this. Firstly, it allows plants which grow well over a range of phosphate levels to be identified. If the phosphate response curves for different plants intersect their relative performance may be very sensitive to changes in phosphorus level. Secondly, there is not a relevant, single phosphate level. In pastures there is a mosaic of phosphate availability resulting from heterogeneous additions of phosphate as dung and pelleted fertiliser. Furthermore moisture stress will not be uniform throughout the pasture. Selecting a single level of phosphate would introduce further artificiality into the procedure.

The success of this programme requires material with repeatable differences in phosphate nutrition that has adequate heritability for breeding. This goal has been given priority. Some phases of the programme will be delayed until this material is obtained.

It is possible that the material with desirable traits of mineral nutrition may not have the other characteristics necessary for good performance. In that case it will be necessary to transfer desirable characters by breeding and it is assumed that this will be possible, although it is not likely to be a simple task.

PROGRAMME OVERVIEW

The programme will proceed in a number of phases, some of them concurrent. Fig. 1 shows the interrelationships between the phases.

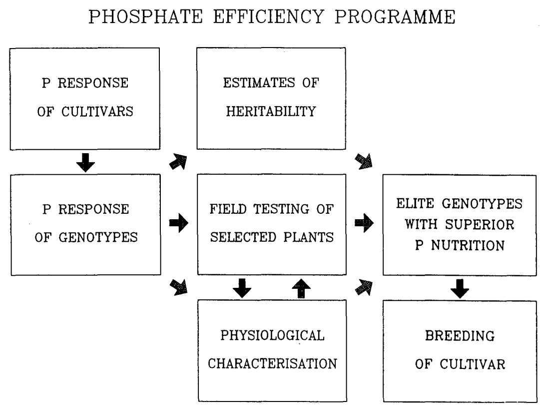

Figure 1: Outline of Grasslands Division's programme to breed a white clover with improved phosphate nutrition.

Stage one — differences in response to phosphate among cultivars. Our first trial established the phosphate response curves for 120 cultivars and lines of white clover which came from a wide range of sources. This stage is discussed in greater detail below.

Stage two — differences in response to phosphate among genotypes. As originally planned this stage was to be a very large pot trial to screen 40,000 seedlings from lines with the greatest differences in response to phosphate. However, the plan was revised after the first experiment. If the variation between cultivars is as large as stage one indicated, the large trial which would strain our resources would be unnecessary. Furthermore we were concerned that the differences found in stage one might be very sensitive to factors in the environment other than phosphate. Therefore stage two has been redesigned. It will provide genotypes with different responses to phosphate and also test whether the order of the differences previously obtained persists under different experimental conditions.

This stage examines the phosphate response of 10 genotypes selected at random from 11 cultivars which were in turn selected for divergent responses to phosphate. An additional 10 genotypes from Grasslands Huia were selected on the basis of the weight of the seedling grown at a single low phosphorus level. The genotypes have been cloned and grown at nine phosphate levels. This trial is presently in progress.

Stage three — heritability of response to phosphate. If the general trend of differences found in stage one is confirmed in stage two the priority will be to determine the heritability of the differences obtained. Heritability will be assessed by determining the phosphate responses of progeny from both a diallel cross and a polycross. There will be 100 seed lines and 10 parents to be evaluated. The experiment will use stolon tip cuttings from 60 seedlings for each seed line to give samples that are representative. The trial will involve 2300 pots.

Stage four — field assessment of genotypes. Concurrent with stage three, a trial will be run to provide information on the field performance of selections from stage two. Some plants with promising phosphate response in stage two may display only mediocre performance in the field. This may be due to factors unrelated to phosphate nutrition which might be corrected by breeding. Alternatively it may indicate that performance in pots when phosphate is limiting does not provide information useful for determining ability to cope with phosphate deficiency in the field. This may be a significant problem, the solution of which will be essential to the success of the programme. However, it is possible that the results themselves may provide insight into the problem.

Furthermore information from stage five, characterising the genotypes should also clarify the problem and help redefine selection criteria.

Stage five — physiology and morphology of the selected genotypes. A further concurrent study will characterise the phosphate nutrition of contrasting genotypes to assess properties which may contribute to good performance at limiting levels of phosphate. In particular characters such as the rate of phosphate absorption, proton extrusion, biochemical partitioning, phosphate distribution within the plant, nitrogen fixation, and the morphology of roots, stolons and leaves will be quantified. Strong correlations between any of these characters and plant performance will suggest that they may equip the plant to cope well under conditions of phosphate stress and might be useful as selection criteria. Experiments to confirm this would be necessary. Determining these characters in the progeny of the crosses of stage three will give information on their heritability and provide a further test of their significance to performance.

The value of any character for screening will depend on its heritability, the extent of its variability and the speed and accuracy with which it can be determined. While screening large numbers of plants for a given character may not be practical, that character may still have value as an additional criterion to assess material selected by other means.

CURRENT PROGRESS

Stage one has been completed. The 120 cultivars and lines that were examined were grown in a glasshouse trial involving 2400 pots. Seedlings were grown at 11 levels of added phosphate, each replicated 1 to 3 times. During growth the pots were watered to weight and rerandomised three times each week. In a single destructive harvest eight weeks after planting leaf number and weight, and stolon length and weight were measured. The trial was labour intensive requiring up to 12 people at the peak periods of planting and harvest. Watering required 16 man hours on each of three days during a week.

A full account of the results has been presented elsewhere (Mackay *et al.,* 1988) and therefore only a summary is given here. There was wide variation between cultivars in their response to phosphate. This was exemplified by the highly significant ($p < .001$) interactions for cultivar x phosphate level for all characters, except proportion of leaf to total shoot weight (Table 1). (The data were log-transformed where necessary to ensure the validity of the assumptions of the analysis of variance.)

Principal component analysis was used to summarise the data. The first principal component, which accounted for 85% of the variation, was a measure of size, with the eigenvectors associated with dry weight measurements, leaf number and stolon length having the largest values. Quadratic response curves fitted to the first principal component gave R values ranging from 89% to 95%. The lack of fit, using the quadratic function, was not significant for 85% of cultivars.

Slopes of the response curves for each cultivar were calculated and cultivars were clustered into eight groups based on rates of response using the flexible-beta method (Lance and Williams, 1967). Fig. 2 shows curves for a cultivar from six clusters. (Three clusters were very similar.)

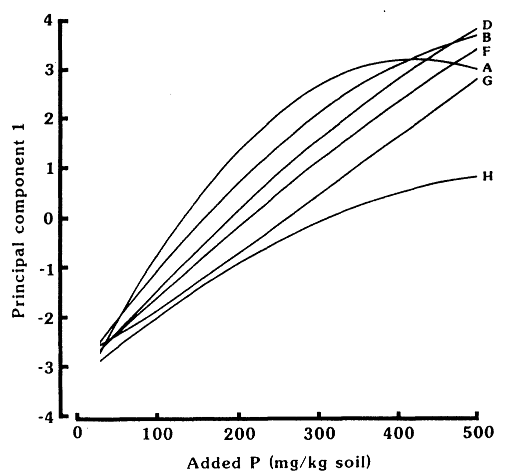

Figure 2: Fitted response curves for example cultivars from cluster A (Gwenda, $y = -3.68 + 0.20x - 0.0015x$), B (Gomelskij, $y = -3.24 + 0.15x - 0.0008x$), D (Podkowa, $y = -3.30 + 0.12x - 0.0004x$), F (Merwi, $y = -3.11 + 0.10x - 0.0002x$), G (Duron, $y = -2.92 + 0.07x - 0.0005x$) and, H (Luclair, $y = -3.28 + 0.09x - 0.0005x$). Curves for clusters C and E were similar to those for cluster F.

There were intersections between curves at both the lower and upper ends of the range of phosphate levels used. The curves show substantial differences in form from markedly convex to slightly concave. The differences in yield indicated are large.

These results are very encouraging. They suggest that there is a large amount of intraspecific variation in phosphate response which might be used in a breeding programme. However, it is possible that some of the differences obtained are subject to factors other than phosphate and it is necessary to first ensure that the differences can, in the main, be reproduced. If they are, the next requirement is that they are heritable. If these two conditions are met there will be good reason for optimism.

CONCLUDING COMMENTS

This programme is not inflexible. Already we have modified it because of the results obtained in stage one. It will be necessary to review the progress made and prospects for success on complete of each stage. This is important not only because we are accountable for the resources being used but also because we are attempting something that has not previously been done and we cannot be dogmatic about the probability of succeeding.

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