

THE PHYSIOLOGY OF PHOSPHORUS AND ITS RELEVANCE TO PHOSPHORUS EFFICIENCY IN WHITE CLOVER

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ABSTRACT

As phosphatic fertiliser is so expensive efforts are being made to improve the efficiency with which it is used by white clover. Part of this effort is a development of an understanding of the physiology of P utilisation after it has been absorbed by the plant. The assumption has been made that some processes are more important than others in determining the efficiency with which P is used in the production of dry matter. This paper is an outline of research, mainly using white clover, on the nature and compartmentation of P compounds in leaves and on the relationship between P and photosynthesis. Some comments will also be made on nitrogen fixation, and on the screening of populations for physiological characters.

Additional Key Words: white clover, phosphorus, nitrogen fixation.

INTRODUCTION

The first thing to do is to establish what is meant by P efficiency. The concept is contentious as it has been found difficult to settle on a definition which suits all occasions. The definition used here applies only to P within the plant, and is used more as an aid to thought than as a universal definition. A phosphorus efficient plant is one which produces the maximum amount of dry matter for a unit input of P; it is also valuable to borrow a concept from economics and to think about a P efficient plant as being one which, at the margin i.e. where revenue (growth) equals expenditure, produces more dry matter for the last unit of P absorbed than the plant with which it is being compared. Plant dry matter is a relatively simple measure of productivity; in the long run, as more knowledge is gained, a more sophisticated measure of productivity may prove to have greater utility. At present, it at least has the virtue of representing net growth of all the plant.

The concentration on internal processes in this review is not meant to deny the importance of P uptake to P efficiency. A distinction between internal processes and uptake is in any case, an artificial one. Physiological processes leading to changes in the partitioning of dry matter within the plant may, for example, have a direct bearing on the volume and area of the roots. (P uptake is dealt with in another paper in this volume: Dunlop).

P is a ubiquitous metabolite; which area of metabolism should be studied? On the face of it at least it seems that in a forage legume some processes may be more important than others in determining P efficiency as defined here. In our laboratory, research has concentrated on the fractionation of P compounds in leaves and on the role of P in photosynthesis. Some research has been done on the P response of white clover provided with mineral nitrogen or reliant on nitrogen fixation, and research is getting underway to look more closely at the relationship between nitrogen fixation and P in nodules. The aim, really, of this

research is to identify those processes involving P which limit the production of dry matter in white clover and hence improve the quality of information we can give to our clients e.g. agronomists, plant breeders, and genetic engineers.

PHOSPHORUS FRACTIONATION

Work on P metabolism has focussed attention on the form of P compounds in plant cells. The concentrations of P_1 , lipid-P, ester-P and residual-P (contains nucleic acid-P) have been measured in leaves of white clover grown at various levels of P supply (Hart and Jessop, 1982, 1983, 1984). Agricultural varieties of white clover may be regarded as competitive — ruderal plants (sensu Grime, 1979) having rapid growth in response to improvements in P supply, which is associated with the accumulation of P_1 in the leaf. The concentration of the other fractions rises with supply but not to the same degree as P_1 . It has been shown in other plants that P_1 surplus to immediate requirements is sequestered in vacuoles and there is some evidence that this also occurs in white clover (Hart, 1985), as is to be expected.

Compartmentation of nutrients within the cell may be extremely important in determining the efficiency with which nutrients are used in metabolism, even to the extent of underlying varietal differences in efficiency. Differences in the relative sizes of the cytoplasmic and vacuolar P_1 pools were observed among cell cultures of *Acer pseudoplatanus*, *Catharanthus roseus* and *Glycine max*, although these could also have been affected by the P_1 supply (Martin *et al.*, 1982). After studying P utilisation in maize, Elliot and Lauchli (1985) concluded that genotypic differences in P_1 accumulation rate may be the result of differences in vacuolar affinity for P_1 . More compelling evidence has been provided by Memon *et al.* (1985b) who showed that three barley varieties which differed in the efficiency of

potassium utilisation differed in the allocation of K between cytoplasm and vacuole.

PHOTOSYNTHESIS

P_i is both a substrate and a control agent in photosynthesis (e.g. Foyer and Spencer, 1986; Sivak and Walker, 1986). In white clover, photosynthetic rate did not decline until the plants were quite deficient and leaf P_i had fallen to low levels (Hart and Greer, 1987). This is presumably a reflection of the ability of P_i in the vacuole to buffer cytoplasmic processes against modest declines in the availability of P. There are limits to this buffering capacity; ultimately the supply falls too low. Other workers have shown that the point where photosynthesis is inhibited by falling P supply, coincides with the vacuoles becoming empty of P (Foyer and Spencer, 1986). As is well known, P deficiency also causes a change in the ratio of starch to hexoses, in the favour of starch, which also inhibits plant growth.

At the P levels that are necessary to give agriculturally worthwhile growth, effects of P on photosynthetic rate per se may not be particularly important. What is important is the leaf area available for photosynthesis. It is well known that area/leaf varies with P supply; this effect was present in our experiment at P levels where there was apparently no effect on photosynthetic rate (Hart and Greer, 1987). If the effect of P on leaf area is not via photosynthetic rate how does it exert its effect? Perhaps through effects on leaf water potential. Radin and Eidenbock (1984) found, in cotton, that low P supply was associated with decreases in root hydraulic conductance and leaf water potential, this being considered to reduce cell size and leaf area. Leaf area is also likely to depend on nitrogen nutrition, carbon acquisition being directly proportional to leaf nitrogen levels (Bloom *et al.*, 1985). In a legume these will be dependent on the activity of the nitrogen fixing system.

NITROGEN FIXATION

The yield of legumes dependent on nitrogen fixation may be lower than that of those provided with mineral N. This difference depends on the P supply; at low P supply yields are similar but at high P levels plants given mineral N are bigger (e.g. Cassman *et al.*, 1980; Hart *et al.*, 1981; Zaroug and Munn, 1979). At low levels of supply both sets of plants are presumably limited by P. At higher levels, the growth of nitrogen-fixing plants may be limited by the ability of the symbiosis to provide fixed nitrogen and by the respiratory burden imposed by the nodules.

Nitrogen fixation requires a lot of P (e.g. Phillips, 1980), and hence the amount of N produced per unit of nodule P becomes of some interest. Nodules contain high concentrations of P (e.g. Hart, 1982) and at low to moderate supplies of P are one of the strongest sinks for P, although this position is not maintained as the supply rises. In an experiment with white clover grown in nutrient solution (Hart, 1989), the roots and shoots came to have concentrations of P as high or higher than those in the nodules as the P supply increased. The activity and

efficiency of the nodules was calculated by growth analysis. The activity (mg N/g/day) of the nodules appeared unaffected by increases in P supply whereas the efficiency (mg N/mg P/day) declined in a manner analogous to the decline in the rate of whole plant growth per unit of internal P. This and findings of other workers (Lynd *et al.*, 1981, 1984; Moustafa *et al.*, 1971) on the concentration of P compounds in bacteroid and cytosol suggests that some of the P in nodules is surplus to immediate requirements to nitrogen fixation. There may be a compartmentation of P analogous to the separation of cytoplasmic and vacuolar P in leaves.

A surplus of P in the nodule would imply that, except at low levels of P supply, nitrogen fixation is not limited by P. If this is correct we are then forced to consider that it may be limited by something else — the rate of arrival of sucrose, the rate of export of fixed N, the rate of photosynthesis or whatever. Ryle and his colleagues (e.g. Ryle *et al.*, 1988) have shown that, in white clover, there is a very close link between nitrogen fixation and current photosynthesis. As was suggested above, in attempting to improve P efficiency, it may be worthwhile attempting to improve the efficiency of processes not directly concerned with P. Once cytoplasmic requirements for P have been satisfied, plants can maximise the return for metabolic expenditure by acquiring something else e.g. N.

SCREENING

It is often assumed that physiologists are able to help in screening genotypes for physiological characters, the idea being that these characters will be a sure guide to performance or that, given their presence is necessary for productivity, they can be transferred to otherwise desirable genotypes currently lacking them. These notions are persistent because of their attractiveness but are fraught with difficulties. Some of the most well known of these difficulties can be grouped under the description 'genotype x environment interactions' e.g. the ranking of genotypes grown in the glasshouse may not coincide with the ranking obtained in the field (e.g. Caradus and Snaydon, 1986; Lawrence, 1984).

Another difficulty is the logistics of screening for characters that are at all complicated to measure. We have developed a sophisticated picture of P usage by white clover, even if it is often based on data from other species, but if, for example, we decide that what is really important to efficient P use is the dynamics of the compartmentation of P in leaf or nodule cells, then the prospect of screening even a small number of genotypes for this character is daunting, let alone the numbers often dealt with in plant breeding trials. We are forced then to turn to less sophisticated but quickly measured characters and hope they will lead us to the desired endpoint. (It is important to note that interpretation of simple characters requires a background of more sophisticated knowledge.)

Some initial work of this kind has been done with white clover. Populations from soil differing in P availability could be characterised by the levels of P_i in their

leaves (Caradus and Snaydon, 1987). Hart and Colville (1988) looked at a number of characters in ten white clover genotypes; the ratio of P_i to P_t appeared to have promise as an indicator of response to P.

Once our understanding of plant function has advanced to the point that it becomes clear that particular characters determine P efficiency, then the techniques of recombinant DNA technology may enable metabolism to be altered fairly directly. For example, the transport properties of the tonoplast and plasmalemma appear to be different (e.g. Butcher and Evans, 1987; Giannini and Briskin, 1987). If it turns out that transport proteins in these membranes have an important role to play in compartmentation, manipulation of them may have desirable effects on nutrient efficiency. Such an approach could avoid some of the disadvantages of classical plant breeding: screening for a superior example of the trait at issue, attempting to transfer the superior trait and finding that other less desirable attributes are linked to it. Recombinant DNA technology offers the opportunity of specific transfer without transferring unwanted genes, although the possibility of unwanted effects on other parts of the plant's metabolism needs to be carefully considered.

CONCLUSION

A study of the physiology of P use enables:

- definition of P efficiency in terms amenable to e.g. economic or thermodynamic analyses. This can give fresh and incisive insights into plant function.
- identification of those areas of metabolism likely to limit P intake and use under various conditions. Human intervention can then be concentrated in those areas.
- prediction of the consequences of manipulation either by classical plant breeding or genetic engineering.

In general, I believe that more understanding is better than less, and that we will be able to improve our advice to plant breeders and come closer to the goal of developing "sampling methods of sufficient convenience, resolution and predictive power to distinguish between genotypes differing in P response" (Hart, 1986).

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