VARIATION IN NITRATE REDUCTASE ACTIVITY AND ITS RELATIONSHIP TO PROTEIN PRODUCTION IN B. NAPUS

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Definition of the biochemical pathways leading to a final plant character offers the possibility of finding one step whose rate of reaction is closely related to the expression of the final character. If the rate of this reaction can be measured simply and rapidly in a non-destructive manner and it can be shown to be a heritable trait, then it should be valuable as a technique for rapid selection in the early generations of a breeding programme.

Where protein is the final character, nitrate reductase activity (NRA) has been suggested as such a selection criterion. In most non-legume crop plants the major supply of nitrogen for the plant is nitrate from the soil. The first step in incorporating this nitrogen into any organic compound is to reduce it first to nitrite and then to ammonia; the ammonia is then immediately incorporated into an amino acid. Nitrate reductase is the enzyme responsible for the first of these reduction steps and is apparently the rate-limiting one since none of the intermediates between nitrate and amino acids are found to accumulate in the plant. A rapid in vivo assay for NR has been developed (Klepper, 1974) and the purpose of the experiments described here was to use this assay to see if NR would be a useful predictor of genetic differences in protein production in rapeseed.

Briefly, the assay involves cutting leaf discs into an incubation medium containing a phosphate buffer, nitrate and a surfactant, vacuum infiltrating, incubating at 33°C in the dark for an hour and then measuring the amount of nitrite present by reacting it with a dye and reading on a spectrophotometer. Activities are expressed as um NO_2^-/g fwt/hr. In fact, since this is an in vivo assay, other factors such as the ability to supply energy for this step will also be measured but this is thought to be a better indication of the in situ activity than NRA alone.

Experiment 1 The first objective of the experiment was to check that the NRA measured is actually reflected in the total assimilation of reduced nitrogen. The second objective was to determine if an early and single sampling date could be related to total seasonal activity. Plants of one cultivar, Tower, were grown in a controlled environment in an inert physical medium with nutrients supplied in solution. Three levels of nitrogen (7, 28 and 112 ppm N as NO_3^-) provided the means of varying the NR levels. Nitrate reductase activities and the reduced nitrogen content (total N less NO_3^--N) were measured weekly in each above ground plant part. Total activity for the life of the plant was estimated by integrating the area under the curve of activity/plant part against time and adding over all plant parts. This value was reflected in the accumulation of reduced nitrogen (Fig.1). The reduced nitrogen is expressed as a percentage in order to remove the dependence of both variables on dry matter production which also varied proportionally to nitrogen treatment. The weekly average NRA up to anthesis also showed a good relationship with the total accumulation of reduced nitrogen (Fig.2).

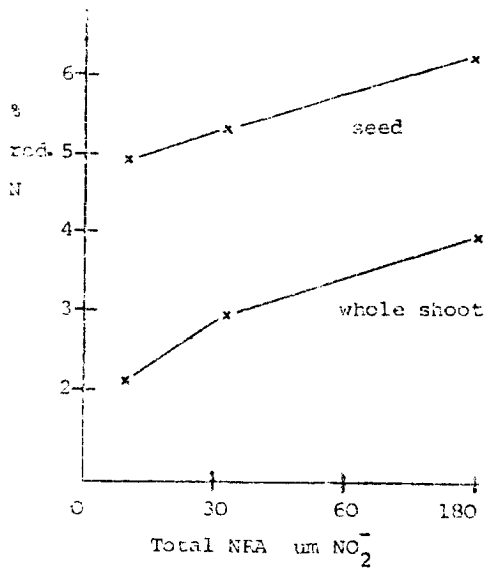

Fig. 1

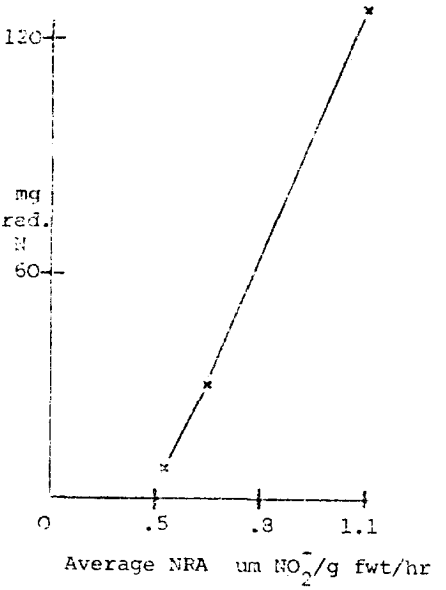

Fig. 2

Levels of NRA for individual leaves showed a distinct pattern which was similar at all nitrogen levels (although the levels were different). This pattern suggested that, provided that sampling is done at an early and comparable stage of leaf growth, any leaf could be chosen to estimate total plant activity.

Experiment 2. The objective of this experiment was to determine whether there is genetic variation in NRA as measured at a single early sampling. Twenty-two spring B. napus cultivars were grown in four replicates in a controlled environment at 25°C and 27 cultivars in a glasshouse during spring. In both cases nutrients were supplied in solution and NRA measured on the day when the first leaf on most plants measured about 15 mm in length. Differences between cultivars were highly significant in both experiments. The results are summarized in Table 1.

TABLE 1

	Controlled environment	Glasshouse
Mean NRA	6.21	2.93
cv %	21.3	21.2
Range of cultivar means	4.15 - 8.00	1.95 - 4.79

The much lower activities measured in the glasshouse are most likely due to the lower temperature; the rate of development was observed to be much slower in this location. The same set of 27 cultivars was also sampled in the field when the plants were at the four leaf stage. Although several sunny days had preceded the two sampling days, activities were very low, sampling variation was high and cultivar differences were not significant. An early sampling date had been chosen to ensure a valid comparison between cultivars which might differ in their pattern of leaf production; although a later sampling date would occur when temperatures, growth rates and NRA

would be higher it was felt that it would be very difficult to make valid comparisons between cultivars when their leaves are at a range of stages.

Experiment 3. The aim of this experiment was to test whether the cultivar differences in NRA determined in Experiment 2 could be related to differences in nitrogen accumulation and protein production in the same cultivars. The plants were grown in a controlled environment until anthesis when half the plants were harvested and analyzed for reduced nitrogen content of the leaves and stems. The remaining plants were transferred to a semi-controlled environment until maturity when seed protein content was measured. Correlations between average NRA values from Experiment 2 and the results of this experiment show that the content of reduced nitrogen in the leaves at anthesis was the only character to be related to NRA (Table 2). However, NRA values for the same 22 cultivars were correlated with protein content from a recent field trial.

TABLE 2

	% reduced N at anthesis		% protein in seed	
	in leaves	in shoot	controlled	field
Correlation with NRA	+.67**	+.11	-.20	+.53*

The content of reduced nitrogen in the leaves was actually significantly negatively correlated with reduced nitrogen in the stem and this could affect the pattern of retranslocation of nitrogen to the developing seed. Although this experiment was unreplicated, the reduced nitrogen content of the leaves ranged from 3.69 to 4.69% and of the whole shoot from 2.86 to 3.37%; this indicates that there are genetic differences in ability to accumulate nitrogen.

Very strong correlations between NR and final protein are probably not to be expected since genetic variation in nitrate uptake, in photosynthetic efficiency and in retranslocation efficiency, will all also affect protein content and protein yield. However, a high NRA does ensure a high genetic potential for providing reduced nitrogen.

REFERENCE

Klepper, L., 1974. A Mode of Action of Herbicides: Inhibition of the normal process of nitrite reduction. Nebraska Res. Bull. 259.