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Title: NITRATE REDUCTASE ACTIVITY IN LEAVES AND ITS RELATIONSHIP TO NITROGEN YIELD IN FOUR SPRING BARLEY (HORDEUM VULGARE L.) CULTIVARS.

Abstract approved: Redacted for privacy

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The main objective of this study was to determine the relationship between nitrate reductase activity (NRA) in leaves and grain yield and protein production in four spring barley cultivars. Differences in NRA in response to three N-fertilizer levels, "enzyme efficiency" and "transport efficiency" were also investigated.

Four spring barley cultivars and three levels of nitrogen fertilizer were the treatments applied. The experimental design was a split plot with four replications in which main plots were fertilizer levels and sub-plots were cultivars. F_2 seed of crosses between selected cultivars used in the main experiment were planted in the field and greenhouse with the same main objective as the parents. The field experiments were conducted at the East Farm Experimental site near Corvallis, Oregon. NRA was assayed at tillering, stem elongation, flowering, grain filling and maturity stages of development. The F_2 individual plants were measured for NRA at the grain filling period only.

Nitrate reductase activity increased with increasing levels of N-fertilizer; significant differences among cultivars were detected. Decreased NRA was observed during stem elongation with an increase noted at flowering and grain filling stages. This latter increase may reflect a greater demand for DNA, RNA and nitrogenous compounds necessary for pollination, fertilization and grain development.

Nitrate reductase activity showed varying degrees of association with nitrate nitrogen (ppm), reduced nitrogen in plant tissue, grain per hectare, percentage of grain protein and total grain protein per hectare. In general the highest R^2 values were observed at stem elongation and flowering. A daily input of reduced nitrogen (DIRN) based on NRA and above ground vegetation was determined which was used to calculate a theoretical input of reduced nitrogen throughout the growing season. Different levels of "enzyme efficiency" among cultivars may compensate for differences in enzyme activity. Cultivars showed differences in "transport efficiency" of nitrogenous compounds from the vegetative parts to the developing kernel.

Daily input of reduced nitrogen showed a higher degree of association with yield and grain protein than NRA. A stepwise regression analysis was run including NRA or DIRN at stem elongation, "enzyme efficiency", "transport efficiency", yield of grain and grain protein production per area. It demonstrated that NRA or DIRN and "enzyme efficiency" were the most important of the above factors that contributed to grain yield and grain protein production per hectare.

Glutamine synthetase activity at the grain filling stage differed among cultivars and in the same cultivar with levels of N-fertilizer

applied. Apparently the activity of this enzyme during grain filling is not directly associated with the agronomic performance of the cultivars studied.

The association between NRA and yield and grain protein in individual F_2 plants grown in the greenhouse and field showed considerable variation among crosses. This was attributed to the late stage of plant development in which plants were sampled and NRA determined, and to the low number of plants sampled.

It was concluded that NRA or DIRN could be useful tools in breeding for yield and protein production in barley. However, there are some limitations to their use in normal breeding programs such as facilities and time for NRA or DIRN assays.

Nitrate Reductase Activity in Leaves and Its Relationship to Nitrogen Yield in Four Spring Barley (*Hordeum vulgare* L.) Cultivars

by

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Typed by Janeil Olsen for Claudio Lovato

IN DEDICATION TO

My wife, Gilca, for all her sacrifices

and

to The Cereal Team

from whom I have learned so much

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I. INTRODUCTION

Plant breeders and scientists concerned with food production are under constant pressure to increase productivity per hectare as a result of expanding population and decreases in available resources.

For many years the only selection tool for increasing grain yield was through yield trials conducted in late generation. The discovery and use of genes for dwarfism and day-length insensitivity represented a major step toward increasing grain yield. It was possible to identify a morphological and a physiological trait that when combined, resulted in a superior germ plasm.

However, it appears that a yield plateau was achieved by several important crops and new breakthroughs in productivity will be difficult to obtain using the traditional methods of selection.

Since grain yield is the end result of several physiological processes in the plant which are regulated by different enzymes, substrates and environmental conditions, it has been proposed that a "biochemical criteria" be developed for plant breeding. According to this proposal the level of activity of certain enzymes could be used as selection tools in breeding for higher yields.

In proposing a strategy for such a criteria two initial steps are very important: first, a proper criterion must be identified; second, the degree of association between the criterion and the final product must be established.

Nitrate reductase activity has been studied as a possible breeding criterion. This interest stems from the fact that nitrate is the

most common form of nitrogen available to plants and that this enzyme is considered as the rate limiting step between nitrate-nitrogen in the plant and amino acids. Most of the studies have been conducted using wheat or corn as experimental organisms with promising but variable results. Only a limited number of experiments were done with barley. The importance of this enzyme and the inconsistency observed justify further studies to ascertain whether or not NRA is associated with yield and protein in barley.

The main objectives of this experiment were:

- To determine the relationship between NRA in leaves and grain yield and grain protein production in four barley cultivars and individual F_2 plants of selected parents;
- To determine differences in NRA among cultivars in response to three N-fertilizer levels;
- To determine differences in "enzyme efficiency" and "transport efficiency" among the cultivars;
- To determine whether glutamine synthetase activity during the grain filling stage was associated with yield of grain and grain protein production.

II. LITERATURE REVIEW

The importance of combining improved grain yield with superior grain protein need not be emphasized. Normally plants take up nitrogen from the soil in the form of nitrate, some of which is applied directly to the soil as inorganic fertilizer. Other forms of nitrogenous compounds are converted to nitrate after rapid nitrification by microorganisms when the temperature is above 5° C.

Nitrate reductase is the enzyme responsible for reducing nitrate to nitrite in the plant cell. The importance of this enzyme in plant metabolism stems from the fact that nitrate is the primary form of nitrogen available to the plant and also nitrate reductase is considered the major limiting factor between nitrate and protein synthesis. This last statement is substantiated by the observation that no intermediates are found free in plant tissue between nitrate and amino acids, although theoretically at least six intermediate compounds exist. The reduction of nitrate in green tissues occurs in the cell cytoplasm and is indirectly associated with the oxidation of metabolites derived from photosynthesis. The reaction catalyzed by nitrate reductase involves the transfer of a pair of electrons from NADH, generated via a NAD-glyceraldehyde dehydrogenase and/or a NAD-malate dehydrogenase, to nitrate. (Magalhaes, 1975).

If the role of nitrate reductase in the plant was to be described in a sentence it could be stated that the level of its activity represents the potential input of reduced nitrogen to the plant. Before protein synthesis can be carried out, nitrate must be reduced. It is not possible to synthesize high amounts of structural and reserve

protein without having a high input of reduced nitrogen in the form of amino acids; therefore, a high level of enzyme activity is needed for high productivity. A great deal of research has been conducted regarding the characteristics, location and the factors which regulate the activity of this enzyme. A detailed review has been presented by Beevers and Hageman (1969).

Since the current study was limited to the interrelationship between NRA and grain yield, grain protein, nitrate and reduced nitrogen in four spring barley cultivars, this literature review will be limited to what was observed regarding these associations in cereal grain.

The pioneer work associating NRA with grain yield and grain protein production was done by Zieserl et al. (1963). Four corn hybrids--Illinois 1996 and Hy 2X Oh_7, reported as high yielders and WF_9 X Oh_7 and WF_9 X C 103, known as low yielders, especially at the high populations used. While NRA assays supported the yield expectation for the three plant populations tested, there was no significant difference among the four hybrids in both years the experiment was conducted. However, three years later when two of the above four hybrids were retested (Hy 2 X Oh_7 and WF_9 X C 103), NRA, grain yield and grain protein production were significantly correlated (Schrader and Hageman, 1965).

In another experiment six corn hybrids grown with supplemental nitrogen and irrigation were studied to discern whether enzyme activity was related to yield and protein (Deckard et al., 1973). The N-fertilizer treatments increased leaf blade nitrate concentration which resulted in a corresponding increase in NRA. Enzyme activity of the total leaf

canopy, expressed as seasonal averages or converted into seasonal input of reduced nitrogen, showed a significant positive correlation with grain protein (kg/ha), grain yeild (kg/ha), and total reduced nitrogen in the above ground vegetation and grain and stover at maturity. The highest correlations between NRA and yields of grain and grain protein (kg/ha) were obtained during the stages of ear initiation and development. This suggests that a minimal number of samplings would be as effective as the laborious full season samplings in selecting individual plants or varieties that have high potential for grain yields or grain protein production. In this experiment, "enzyme efficiency" (estimated input of reduced nitrogen based on NRA and the actual input of reduced nitrogen) for the six hybrids used ranged from 2.2 to 4.1. In spite of marked differences in enzyme efficiency of the hybrids, increases in enzyme activity were significantly correlated with increases in grain protein ($r = .90^{**}$ and $.83^{**}$) for the hybrids with lowest and highest enzyme efficiency respectively.

The proportion of reduced nitrogen translocated from the vegetative parts to the grain at maturity and variations in enzyme efficiency may vary among widely different genotypes. Thus the degree of association between NRA and total grain protein production might be affected by these two factors. One experiment with a partial diallel set of corn grown at the University of Illinois, Urbana, was conducted in 1973 to test these two criteria. The correlation coefficients for all 36 hybrids used was .41 (significant) between enzyme activity and grain yeild and .33 (non-significant) between enzyme activity and total grain

protein production. When the hybrids were divided into groups, based on one common parent, some increase in the correlation coefficients was observed in a majority of the groups (cited by Hageman et al., 1976).

The possible relationship between level of NRA to water soluble leaf protein and to yield of grain and grain protein in Ponca and Monon wheat cultivars was investigated by Croy and Hageman (1970). They also examined numerous hard and soft winter wheats grown under standard nursery conditions to determine whether cultivar differences could be detected in NRA and production of grain protein (percentage and total). They showed that: (1) increased enzyme activity from supplemental nitrogen treatments was associated with increases in grain protein, both percent wise and total; (2) a significant correlation was found between the spring seasonal total of NRA (units of nitrogen reduced per hectare) and grain protein (kg/ha) for both varieties; (3) this correlation was valid only for a specified genotype as Ponca required a higher level of enzyme activity to accumulate a unit of grain protein than Monon. For the 32 winter wheat cultivars evaluated under low nitrogen levels, the average values of enzyme activity obtained from three fall and three spring samplings showed little variation among genotypes and no correlation with grain or protein production. However, selection of 16 of the cultivars exhibiting the highest enzyme activity as noted from samples taken on November 12th, when soil nitrate was in adequate supply, would have included nine of the 13 cultivars which ultimately produced higher grain protein.

In another field experiment, Eilrich and Hageman (1973) observed that in field-grown Arthur wheat, the input of reduced nitrogen estimated from NRA of the entire canopy was significantly related to the amount of reduced nitrogen of the above ground vegetation. Since translocation of vegetative nitrogen to the grain was uniform across N-fertilizer treatments, a significant positive correlation was found between NRA (expressed as moles of N/ha per season) and grain nitrogen (kg of N/ha) at maturity. Seasonal NRA also correlated significantly and positively with grain yields. Similar results were obtained with Ottawa wheat.

Eilrich (1968) (cited by Hageman et al., 1976) examined the influence of genetic diversity upon the association between anzyme activity and grain nitrogen in 14 wheat cultivars. Ten were related genotypes from a cross of Atlas 66 X Comanche while the other four were of diverse origin. The correlation coefficient between seasonal enzyme activity and grain nitrogen (kg/ha) for the ten related cultivars was significant ($r = .57$), in contrast to a non significant, $r = .38$, when all 14 varieties were compared. Hageman et al. (1976) stated that this study again revealed that "transport efficiency"--that is, the percentage of nitrogen retained in the straw, was a function of the genotype, and "enzyme efficiency" (the ratio between calculated input of reduced nitrogen from enzyme assay to actual input) could affect the correlation between enzyme activity and yield of grain or total grain protein.

Data obtained with five Australian wheat cultivars that differed in date of maturity, vegetative mass, and grain protein, showed a

significant relationship between input of reduced nitrogen estimated from nitrate reductase assays and actual vegetative nitrogen from seedling to booting stage. A significant correlation was also noted between seasonal input and actual nitrogen in the straw and grain at maturity with all five cultivars. However, a significant relationship between enzyme activity and grain nitrogen was not observed without taking consideration of transport efficiency. When the cultivars were grouped according to the ratio of grain nitrogen to total plant nitrogen (translocation efficiency), a very significant relationship was observed between the enzyme activity and grain protein production (Dalling, et al., 1975).

The value of NRA as a selection tool would be greatly enhanced if non-destructive evaluations could be done at an early stage of plant development. Zeiserl et al. (1963) and Warner et al. (1969) observed that in corn, seedling stage NRA reflected the level of activity throughout the season. Other experiments have demonstrated that enzyme activity in the field is paralleled with activity in the growth chamber (Cray and Hageman, 1970: Samphantharah, 1973). Therefore, seedlings grown in a growth chamber would be useful for this kind of study.

The relationship between enzyme activity in wheat seedlings grown in the field at one location (Lincoln, Nebraska) and the percentage of grain protein of comparable genotypes grown in the field at another location (Yuma, Arizona) was investigated (Hageman et al., 1976). The following procedure was used to select the 53 genotypes tested. Each lot of F_2 seed in the original group was obtained from individual plants

that were selected for high grain yield and desirable morphological traits. From this group of genotypes, a second selection was made for F_2 seeds with high protein. This process was repeated in the F_3 generation, with selection being made first for yield and morphology and second for protein, to provide the 50 genotypes (F_4 seed) used in the experiment. The three control entries were Atlass 66, Inia 66, and Nap Hal. Although the correlation was not significant, there was a distinctive trend associating enzyme activity in one location at seedling stage with percent protein in the grain at a second location, suggesting that NRA has potential as a selection criterion for developing high protein wheat cultivars.

A study in wheat compared the class distribution (based on levels of NRA) of 97 F_2 genotypes with the class distribution of 87 parental lines. The objective was to observe whether normal selection procedures also discard the low nitrate reductase lines (cited by Hageman et al., 1976). In vivo enzyme assays were made with 3,546 individual F_2 seedlings and with replicated bulked samples of the 87 cultivars. A total of 896 individual F_2 seedlings (25 percent) had activity lower than any variety. The greatest number of F_2 seedlings fell in the 2-3 class range (moles NO_2 reduced gfw/hr) while the greatest number of parental lines fell in the 3-4 class range. The absence of the low (1-2) class range among the parental lines suggests that the normal selection procedures used in selecting the parental lines also discarded the low nitrate reductase lines. In contrast only 24 of the 3,546 individual seedlings (.67 percent) had activities higher than the

highest parental lines. This indicates that new cultivars could be selected from this genetic material with higher levels of NRA.

Nitrate reductase is a substrate inducible enzyme. However, the correlations between level of enzyme activity and nitrate content of the leaf tissue have been variable. In corn, Zieserl et al. (1963) observed that intitial nitrate content of the leaf tissue was very high but dropped rapidly during vegetative development. There was a negative correlation between nitrate content and specific enzyme activity, suggesting that nitrate reductase was affecting the reduction of nitrate in the leaves. In another experiment with corn (Deckard et al., 1973), enzyme activity was significantly correlated with leaf nitrate concentration only for a limited period after silking. The most probable cause for the lack of correlation prior to silking was that the level of nitrate in the leaf blade was in excess of the minimum level needed for expression of the genetic potential for synthesis and maintenance of nitrate reductase. In wheat NRA was positively correlated with nitrate content of leaf tissue (Eilrich and Hageman, 1973; Croy and Hageman, 1970).

The accumulation of reduced nitrogen by the above ground portion of the plants during growth and increase in reduced nitrogen due to nitrogen fertilizer is attributed primarily to NRA. Eilrich and Hageman (1973) observed in wheat a significant correlation between NRA (input estimated as moles N/ha for a given period) and the measured increase in total reduced nitrogen (kg N/ha) in the vegetative tissue during the same period. In their experiment the ratio of input of reduced nitrogen as estimated by enzyme assay to actual accumulation of reduced nitrogen

in the stems and leaves varied considerably according to the period in which they were calculated suggesting that in vitro nitrate reduction does not always correlate with in situ nitrate reduction.

The relationship between NRA and yield and protein has not been studied as extensively in barley as in wheat or corn. Samphantharah (1973) studied the physiological aspect of nitrate reductase and grain protein in six barley cultivars--three considered as high protein and three considered as low protein cultivars. Seeds were space planted with the fertilizer being applied in the form of calcium nitrate at a rate of 136 kg/ha of nitrogen. The cultivars with low percent protein showed a higher enzyme activity per gram of fresh weight of leaf tissue than those cultivars with higher percent protein. This was observed at all stages of growth except the cultivar Nordic which expressed low enzyme activity at the early stages of development. When the cultivars were grouped according to their enzyme activity at 20 days, the high enzyme activity group had a higher total grain protein than the lower enzyme activity group. A significant correlation between NRA and water soluble protein in leaves at both tillering and booting stages was also observed.

Besides showing a reasonable relationship to productivity or quality of the product, any breeding criterion should be highly heritable. Evidence suggests that the level of NRA is highly heritable. In corn it was observed that F_1 hybrids obtained by crossing two inbreds lines with high enzyme activity were high in activity but no higher than the parental inbreds. Crosses between parents with high and low activity

produced hybrids with intermediate activity. Hybrids from inbreds low in activity possessed either low or heterotic level of activity. This superior ability to reduce nitrogen was observed in all stages of plant growth (Schrader et al., 1966).

The enzyme activity inheritance pattern in corn was studied by Warner et al. (1969). They demonstrated that two inbreds (B_{14} and Oh_{43}) differed at two loci that control NRA. One locus appeared to affect the rate of enzyme synthesis while the other locus altered the rate of decay of the enzyme. The data suggested that the heterotic level in F_1 resulted from the inheritance of qualities that give "intermediate" rates of enzyme synthesis and decay.

General and specific combining abilities were estimated for a diallel set of crosses using ten parents and 45 corn hybrids (cited by Hageman et al., 1976). Both general and specific combining ability effects were important. The general combining ability mean square was about three times greater than specific combining ability mean square. The magnitude of general combining ability effects relative to specific combining ability indicates that selection for increase NRA in corn should be effective.

Any breeding criterion must exhibit genetic variability among genotypes of a same species. Nitrate reductase activity varies considerably among genotypes. Variations up to fivefold were observed in corn inbreds (Zeiserl and Hageman, 1962); twofold for corn hybrids (Zieserl et al., 1963); twofold for Sudan grass (Eck and Hageman, 1974); twofold for soybeans (Harper et al., 1972). Therefore, according to

these authors, NRA has the potential to be used as a breeding tool in the improvement of crop productivity and protein yield.

III. MATERIALS AND METHODS

Three separate experiments were conducted. Experiment I was primarily designed to discern:

a. The differences in NRA in response to three N-fertilizer levels.

b. The variation in NRA at several stages of plant development.

c. The relationship between NRA and nitrate and reduced nitrogen in the plant, yield of grain and grain protein production.

d. The differences in "enzyme efficiency" and "transport efficiency" among the cultivars studied.

e. The association between glutamine synthetase activity during grain filling and yield and grain protein production.

Experiment II was conducted to investigate the relationship between NRA in leaves during the grain filling stage, with grain yield and protein of individual F_2 plants when grown under field conditions. Experiment III was conducted in the greenhouse with the same objectives as in Experiment II, and also involved F_2 segregating plants.

In Experiment I the experimental design was a randomized split plot with four replications. The treatments consisted of four spring barley cultivars and three levels of nitrate nitrogen fertilizer. Cultivars were sub-plots and nitrogen levels main plots. The cultivars Larker and Karl are malting barleys where low grain protein is desirable. Steptoe and Benton are regarded as feed barleys in which higher protein content is an advantage.

The nitrogen treatments were: control (0 kg of N/ha), 60 kg of N/ha and 160 kg of N/ha applied in the form of calcium nitrate. In the

highest nitrogen treatment, 100 kg of nitrogen per hectare was incorporated prior to seeding and 60 kg of N/ha was top-dressed at the intiation of the reproductive stage. In the control and 60 kg of N/ha treatments the fertilizer was incorporated to the soil prior to seeding. The objective of the split application (100 60 kg of N/ha) was to maintain a high level of available nitrate nitrogen during all stages of plant development and thereby increase NRA. All plots received 60 kg/ha of potassium in the form of potassium chloride and 100 kg/ha of phosphorus in the form of sulfur phosphate (P_2O_5). Both fertilizers were incorporated into the soil during seed bed preparation.

The experimental site was located on the East Farm near Corvallis, Oregon. The soil type at the East Farm is the Newberg series which is a fine sandy loam soil. The experimental area had been previously fallowed for two years. Seeds of the selected cultivars were planted on April 15, 1977, at a rate of 20 kg/ha with a V-belted seeder.

The seeds were pretreated with the systemic fungicide Vitavax (5-6-Dihydro-2-methyl-1, 4-oxathiin-3-carbonanilide) at the rate of 185 grams for 100 kg of seeds, to prevent loose smut (Ustilago nuda). All plots were irrigated in order to enhance germination one week after seeding. Irrigation was also used after top-dressing with nitrogen and as necessary during the developmtn of the plants to avoid moisture stress. Irrigation during the grain filling period caused moderate to severe lodging in 16 of the 48 sub-plots. To decrease the effect to lodging, wooden frames were built and placed 50-70 cm above the ground

with strings criss-crossed in two directions to maintain the plants in erect position. Each sub-plot was 5.3 m long and consisted of six rows spaced 30 cm apart. Weeds were controlled by hand hoeing. The fungicide Benolate (Mathyl-1 (butylcarbonioyl)-2-benzinidazole) was sprayed with water at the rate of 2 kg/ha of active ingredient, in order to control powdery mildew (Erysiphe graminis).

In Experiment II the experimental design was a randomized block with three replications. All plots were fertilized with nitrogen (140 kg/ha) phosphorus and potassium as in the adjacent Experiment I. Seed treatment with Vitavax, seeding rate, weed control, irrigation and Benolate application were the same as in the main experiment. The date of seeding was April 21, 1977.

Experiment III was conducted inboth the growth-chamber and greenhouse. F_2 seeds were seeded in small plots, allowed to germinate and then placed in a growth-chamber. The growth-chamber was used to increase number of tillers per plant as in the month of July high temperatures and long photoperiods limit tillering. A night temperature of 15^{o} C and day temperatures of 24^{o} C were established. The photoperiod was set to 16 hours of dark and eight hours of light with a light intensity of 2000 footcandles. After four weeks in the growth-chamber, plants were transplanted to larger pots and then moved to the greenhouse. Temperatures were variable in the greenhouse with highs up to $30\text{-}32^{o}$ C depending upon outside temperatures. Supplemental light was used. The photoperiod was 16 hours of light per day and light intensity was 2200 footcandles. The initial seeding date was July 10th.

I. Sampling Procedures

A. For NRA:

Samples to assay NRA in Experiment I were taken at the stages of tillering, stem elongation, flowering, seed development and maturity. Since variation in NRA exists depending upon the position and age of leaves (Harper et al., 1972), the leaves known to contain maximum activity were therefore selected for samples. Newly fully developed leaves (before flag leaves formation) and flag leaves (after their formation) were collected from the four external rows of a plot because the two middle rows were reserved for grain yield and the percentage of grain protein.

Samples were collected between 1:00 and 1:30 p.m. since there is a diurnal variation in NRA (Beevers and Hageman, 1969). For the individual segregating plants in Experiments II and III, only one sampling was done and this was at the time of grain filling. The two leaves immediately below the flag leaves were collected. For Experiment I, 30 to 35 flag leaves were randomly selected from each sub-plot. These were folded in a moist paper towel, placed in a cooled styrofoam ice chest and immediately brought to the laboratory for analysis. In order to avoid the effects of enzyme denaturation over time, only one complete replication was done per day since nitrate reductase assay is a relatively time consuming procedure. Therefore, replications were assayed at different days.

B. Glutamine synthetase activity:

Flag leaves were sampled for glutamine synthetase at the stage of grain filling. Assays were conducted among the four cultivars within

the 160 Kg/Ha of nitrogen treatment and for the cultivar Benton, under the three nitrogen treatments. Number of leaves sampled, time of sampling, and sample storage were the same as in Experiment I.

C. Weight of above ground vegetation:

In each stage of development in which NRA was assayed, total weight of the above ground vegetation was sampled. Row segments 60 cm long were collected from each sub-plot, weighed, dried in an oven at 70° C. for 48 hours and then weighed again. The values obtained were used to calculate fresh and dry weight of the above ground vegetation of each sub-plot and stage of growth. These same samples were kept and later used to assay nitrate nitrogen and total nitrogen in the plant tissue.

D. Grain yield and percentage of grain protein:

Samples for grain yield and percentage of grain protein were taken by harvesting the two central rows of each sub-plot. The length of each row collected was three meters.

II. Methods of Analysis

A. Nitrate reductase activity:

The nitrate reductase assay used in this experiment was a modified in vivo method of Streeter and Bosler (1972). Nitrate reduction involves the transfer of two electrons from NADH generated from the oxidation of 3-phosphoglyceraldehyde to 1, 3 diphosphoglyceric acid by 3-phosphoghyceraldehyde dehydrogenase.

In the leaf tissue the nitrate transferred from the roots to the cytoplasm of the cell is reduced by nitrate reductase to nitrite with

NADH as the reductant. Nitrite then enters the chloroplast. In the chloroplast nitrite is reduced to ammonia by nitrite reductase. This reduction is linked to the light reactions of photosynthesis which provide reduced ferredoxin as a source of electrons for the reduction process. The incorporation of ammonia into the α-amino group of amino acids also occurs in chloroplasts. Two enzymes are involved in the synthesis of amino acids and amides: glutamate dehydrogenase and glutamine synthetase. In the in vitro nitrate reductase assay, the enzyme is first isolated and an external source of NADH is used to drive the reduction process. In the in vivo method, the enzyme is not isolated. NADH is derived from the glycolysis of sugars which are formed by the photoshythetic process. The reaction is conducted in the dark to prevent photosynthesis, thus avoiding further reduction of nitrite to ammonia. The enzyme activity is measured by the amount of NO_2 formed after certain time per gram of leaf fresh weight. The reaction is also conducted under anaerobic conditions to avoid the oxidation of NADH in the respiration process. Although the enzyme activity assayed by the in vivo method is lower than the activity detected with the in vitro method, both are highly correlated (Streeter and Bosler, 1973) and probably better resemble the in situ conditions (Klepper,1974).

General Description of the in vivo Method:

Reagents:

(a) Substrate buffer: 0.05M KH_2PO_4 0.1M KNO_3 pH 7.5

(b) Reaction buffer: 0.05M KH_2PO_4 pH 7.5

(c) Sulfanilamide 1% in 1.5M HCl to stop the reaction.

(d) Color reagent: 0.02% of N-(1-naphthyl) ethylenediamine dihydrochloride.

Procedures:

1. Five ml of reagent (a) were placed in three of 30 ml beakers, and five ml of reagent (b) in two other beakers. All beakers were placed in an ice bath to retard the reactions until all material was ready and transferred to the incubation bath.
2. Only the middle portion of sample leaves was used in analysis and both extremities were discarded. Sections 3-4 mm long were cut with a razor blade, well mixed, and 0.2 grams placed into each of the five beakers. A small stainless steel screen was placed over the leaf pieces to keep them under the solutions.
3. The beakers were transferred to a tray which was placed in a dissecator linked to a vacuum pump. Partial vacuum (640 mm Hg) was applied for three consecutive periods of one minute each.
4. The tray was retrieved from the dissecator, covered with aluminum foil and incubated in a water bath at 30^{o} C. with constant shaking for ten minutes.
5. At the end of ten minutes the tray was removed from the incubation bath and placed in ice. Leaf segments were removed and the reaction stopped by adding 2.5 ml of reagent (c) to all beakers. To two of the beakers without substrate 100μ L of NO_2 (1 mM concentration) was added for a "standard". Then 2.5 ml of color reagent (d) was added to all beakers.
6. A "blank" was also prepared by using the same procedures as for the "standard", but without leaf fragments to eliminate endogenous nitrite which may vary from sample to sample.
7. After a minimum of 15 minutes to insure complete color development, absorbance of the solutions was read at 540 mm against a water blank.

8. Calculations: moles of NO_3 reduced per hour per gram of leaf fresh weight =

$$\frac{\text{Absorbance sample}}{\text{Absorbance standard}} \times \frac{.1}{.2} \times \frac{60}{10}$$

B. Glutamine Synthatase Activity

Because little glutamate dehydrogenase activity has been found in leaves and high glutamine synthetase activity is detected, this enzyme is believed to be responsible for most of the ammonia incorporated into various amino acids. It binds an amino group to glutamate forming glutamine which functions as a reservoir of ammonia in plants. Another enzyme, glutamate synthetase, would then transfer one amino group from glutamine to α-ketoglutaric acid to form glutamate. By transformation, which involves the transfer of an amino group of an amino acid to the carboxyl group of a keto acid and other synthetic pathways, different amino acids are formed.

Procedures for Glutamine Synthetase Determination:

The method followed for glutamine synthetase activity determination was the one proposed by O'Neal and Joy (1973).

Isolation of the enzyme:

Isolation of the enzyme was carried out inside a cold room. Each sample was divided in two sub-samples of one gram each. The central portions of the leaf were used. The leaf fragments were ground in a mortar with pestle using a total amount of ten ml of grinding buffer per sub-sample. The composition of the grinding buffer was:

HEPES	0.1 M
$Mg(Ac)_2$	0.004 M
Sucrose	0.1 M
450 ml of H_2O	pH was adjusted to 7.5

with KOH; water was added to make a total of 500 ml. The grinding buffer was stored in a cold room and prior to use 0.08 ml of mercaptoethanol per 100 ml of grinding buffer was added. During grinding, one ml of PVP slurry (1 gr/10 ml of grinding buffer) was added to the mortar. The extract was transferred to a plastic centrifuge tube and centrifuged at 0° C. and at 18,000 rpm for ten minutes. The residue was discarded and the supernatant collected as the enzyme preparation. The top fat layer that appears after centrifugation was removed with a piece of absorbant paper.

Enzyme Assay:

(a) Reagents:

Reaction buffer containing:

HEPES	0.1 M
M_gSO_4	0.04 M
Glutamic acid	0.16 M, pH 7.5

(b) Energy source:

0.02 M ATP was added to reagent (a). For ten samples 130 mg of Na_2 ATP was added to 10.4 ml of (a) plus 0.1 ml of 2 N KOH.

(c) Second substrate:

Hydroxylamine was added to reagent (a). For 15 samples 69.5 mg of NH_2OH was added to 4.6 ml of reagent (a), plus 0.4 ml of 2N KOH. Prior to use 0.16 ml of mercaptoethanol was added to 100 ml of reagent (a) which had been stored in a cold room.

(d) Color reagent:

$FeCl_3.6H_2O$	0.18 M
HCl	0.67 M

Trichloroacetic acid 5%

Volume completed with H_2O to 1000 ml.

Procedures:

1. A "blank" was prepared by adding 0.5 ml of reagent (a).
2. 0.5 ml of reagent (b) was pipetted in three tubes for each sub-sample.
3. 0.4 ml of enzyme preparation was pipetted to the above four tubes.
4. All tubes were incubated at 37° C. for two minutes.
5. 0.1 ml of reagent (c) was added to all tubes, well mixed, and incubated at 37° C. for ten minutes.
6. One ml of color reagent (d) was added to each tube, mixed well and let stand for ten minutes.
7. One ml of H_2O was added to all tubes. Tubes were centrifuged at 5000 rpm for one minute.
8. Within 15 minutes absorbance of the supernatant was read against the respective blank at 540 nm.
9. μ moles of glutamyl hydroxymate produced in ten minutes was calculated by:

$$X = OD_{540} \div 0.262 \qquad \left(0.262 = \frac{\text{mM extinction coefficient}}{3}\right)$$

10. Specific activity was calculated by:

$$\frac{X}{10 \times \text{mg protein in 0.4 ml enzyme preparation}}$$

C. Protein in the Leaf Extract:

To express glutamine synthetase activity, soluble protein in the leaf extract was assayed by Lowry's rapid method (Schacterle and Pot-lack, 1973).

D. Total Nitrogen in Plant Tissue and Grain:

Total nitrogen in plant tissue and grain was determined by the Kjeldahl method. For grain protein, each sample was run in triplicate and the results averaged. For protein in grain, the percentage of grain nitrogen was multiplied by a constant factor of 6.25.

E. Nitrate Nitrogen in Plant Tissue:

Nitrate nitrogen was determined by "steam distillation method for nitrate determination in plant tissue" used in the Soil Sciences Department, Oregon State University.

Description of the method:

1) Samples were ground and dried at 70° C. for at least 24 hours.
2) One gram samples were transferred to a plastic container to which 50 ml of .002 M Formic acid was added. The containers were shaken for 30 minutes.
3) The extract was filtered through a paper filter.
4) To a distillation flask to which .8 gr of magnesium oxide had been added, an aliquot of 20 ml of the extract was transferred. Distilled water was added up to half the volume of the flask.
5) The flask was placed in the distillator and the first 75 ml of distillate were discarded in order to eliminate any nitrogen in ammonia form.
6) .8 gr of Devarda alloy was added to the distillation flask and 75 ml of distillate were collected in an erlenmeyer flask containing ten ml of boric acid indicator solution.
7) Tritation was done with 0.02 N HCl.
8) Nitrate nitrogen (ppm) was given by:

NO_3N = Factor x (sample-blank)

$$\text{Factor} = \frac{\text{Normality of HCl} \times 14 \times 1{,}000{,}000}{1{,}000 \times \text{sample size (1 gr)} \times \frac{\text{Aliquot size (20 ml)}}{\text{sample extract (50 ml)}}}$$

F. Percentage of Reduced Nitrogen in Plant Tissues:

Percentage of reduced nitrogen in the plant was obtained by subtracting percentage of nitrate nitrogen from the percentage of total nitrogen. Nitrate-nitrogen, total nitrogen and reduced nitrogen were expressed as percentage of plant dry weight.

III-A. Daily Input of Reduced Nitrogen per Hectare:

Daily input of reduced nitrogen was calculated by this formula:

$$D.I.R.N. = NRA \times g.frw/Ha \times 12 \times 10^{-6} \times 14$$

where: NRA = enzyme activity

g.frw/Ha = grams of plant fresh weight per hectare

12 = 12 hr/day - arbitrarily selected on the basis of the known variation in NRA (Deckard et al., 1973)

10^{-6} = to transform μ moles to moles

14 = gram of N per NO_3 mole

B. The Theoretical Input of Reduced Nitrogen per Hectare Throughout The Growing Season:

The theoretical input of reduced nitrogen per hectare throughout the growing season was calculated by integrating the daily input of reduced nitrogen from emergence to maturity. The integration was done by averaging the daily input of reduced nitrogen at two consecutive stages of development in which enzyme activity was measured and multiplying the number of days between them.

C. "Enzyme Efficiency":

Enzyme efficiency was obtained by dividing the theoretical input of reduced nitrogen in Hg per hectare throughout the growing season by the actual accumulation of reduced nitrogen in straw and grain per hectare at maturity (Hageman et al., 1976). The lower the ratio the higher the enzyme efficiency. Enzyme efficiency is therefore defined as the amount of nitrate reductase input to the end product--in this case, total reduced nitrogen per area per season.

D. Percentage of Total Nitrogen per Area Retained in the Straw at Maturity:

Percentage of total nitrogen per area retained in the straw at maturity was obtained by dividing total nitrogen in straw by total nitrogen in straw and grain at maturity. Straw and grain at maturity contain different proportions of nitrogen. Therefore when determining nitrogen in straw, the grain was separated out before grinding the samples.

4 - Statistical Analysis

To determine whether there were significant differences due to the treatments applied, analysis of variance was conducted. Nitrate reductase activity, percentage of reduced nitrogen and concentration of nitrate nitrogen in the plant tissues were initially analyzed as split-split-plots in order to detect differences due to stages of plant development. Each stage of development was also analyzed individually.

For multiple comparison tests, Duncan's new multi-range test was applied. The level of significance chosen was the 5%. However, in certain cases a treatment or interaction would be significant only at

the 10 percent level of significance. In those cases it is indicated in the bottom of the tables. To evaluate the degree of association between NRA and certain plant parameters such as protein, yield, nitrate nitrogen and reduced nitrogen, a regression analysis was conducted between enzyme activity and those parameters. The quadratic model of regression analysis was adopted for it was considered biologically better fitted than other models. From the regression analysis a coefficient (R^2) of multiple determination was obtained. The R^2 measures the proportion of total variation about the mean $\overline{Y}$ (dependable variable) explained by the regression.

A stepwise multiple regression analysis was conducted to assess the contribution of NRA and daily input of reduced nitrogen at the stage of stem elongation, enzyme efficiency and transport efficiency to yield of grain and grain protein production. The stage of stem elongation was chosen because it was the stage that in general showed the greatest degree of association between NRA and daily input of reduced nitrogen with N-yield criteria.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

Plant growth and development require an adequate supply of nitrogen. High levels of nitrate-N in the soil are of limited value if the plant is unable to reduce it in the amounts necessary for maximum yield and protein production. Hence, high levels of NRA in plants are important to productivity. Nitrate reductase is an inducible enzyme and the extent of inducibility varies in different cultivars. In this experiment three levels of N-fertilizer and four spring barley cultivars were used to assess the variability in NRA. However, comparable NRA may not result in equal amounts of protein production in seeds or forages among different cultivars. Both the total concerted effort of other assimilation enzymes and "transport efficiency"of metabolites to grains or forages play an important part infinal productivity. Therefore, "enzyme efficiency" and "transport efficiency" were also investigated in this study.

The results and discussion will be presented as follows: Experiment I: effect of treatments upon yield, protein, nitrate-nitrogen and reduced nitrogenin plant; the effect of treatments upon NRA and the interrelationship between NRA and several N-yield criteria; effect of treatments upon glutamine synthetase activity; contribution of NRA, "enzyme efficiency" and "transport efficiency" to yield of grain and grain protein production.

In Experiments II and III, the interrelationship between NRA and grain yield and grain protein production will be presented.

I-1: Grain Yield:

Grain yield of the four spring barley cultivars was significantly affected by N-fertilizer treatments. No interaction between cultivar and N treatments was observed. Table 1 shows the yield responses of the cultivars to the three N-fertilizer levels.

Table 1. Yield (kg/ha) of four barley cultivars to three nitrogen-level treatments.

N Treatments kg/ha	Cultivars				Average
	Steptoe	Larker	Benton	Karl	
0	3,232a*	2,646a	2,719a	2,529a	2,782c
60	5,558a	4,174bcd	3,874b	4,667ab	4,568b
100 + 60	7,009a	5,168b	5,501b	5,409b	5,772a
Average	5,262a	3,996b	4,032b	4,202b	

* Cultivar means at the same level of N, means of N levels and overall cultivar means, followed by different letters are significantly different (DMR test) at the 5% level of significance.

The nitrogen treatment in which 60 kg of N/ha was applied, increased yields of grain on an average of 64% of the control. Each kg of N-fertilizer produced an average increment of 29.76 kg of grain per hectare. The higher N-fertilizer treatment increased the average grain yield by 107% of the control. Each kg of N-fertilizer for this treatment produced an average increment of 18.68 kg of grain per hectare. In regard to individual cultivars at the 60 kg of N/ha, Steptoe, Larker, Benton and Karl produced an additional 38.76, 25.46, 19.25 and 35.63 kg of grain

per hectare for each kg of N applied as fertilizer respectively. At the 100 + 60 kg of N-fertilizer treatment, each kg of N applied increased yield by 23.60 kg/ha in Steptoe, 15.76 in Larker, 17.39 in Benton and 18.00 kg/ha in Karl.

Nitrogen fertilizer increased the weight of above ground vegetation considerably in this experiment (Appendix 2). It is reasonable to assume that to this increased plant weight per area also corresponded with an increase in Leaf Area Index (LAI). Tsumoda (1972) observed that in wheat and rice, nitrogen fertilizer also increased leaf chlorophyll content and photosynthesis per unit of leaf area. Considerable difference in leaf color was observed for the different nitrogen fertilizer treatments. The nitrogen fertilized plots also matured a few days (5-6) later. Therefore, differences in photosynthetic capacity related to leaf area index, leaf area duration and photosynthesis per unit of leaf area may explain the differences in average yield response to nitrogen fertilizer application. Watson (1956) studied the relationship between LAI and yield of grain in cereals. He observed that high LAI at heading and slow senescence of the leaves, mainly flag leaves, were characteristics favoring high yields.

Differences in yield among cultivars at a same level of nitrogen fertilizer can be attributed to the same causes aforementioned as well as to differences in photosynthate partitioning at maturity.

I-2: Percentage of Protein in Grain:

Percentage of protein in grain was significantly affected by nitrogen fertilizer levels and cultivars. A significant interaction as shown by analysis of variance (ANOVA) table (Appendix 1) between nitrogen

levels and cultivars was detected. Table 2 indicates the percentage of grain protein in four cultivars as influenced by nitrogen fertilizer levels.

Table 2. Effect of three nitrogen levels on percentage of grain protein of four barley cultivars.

N Treatments kg/ha	Cultivars				Average
	Steptoe	Larker	Benton	Karl	
0	8.17b*	9.28a	9.78a	9.08a	9.08b
60	8.48c	9.47ab	10.03a	8.90bc	9.22b
100 + 60	12.31b	14.80a	14.50a	12.50b	13.52a
Average	9.65b	11.17a	11.44a	10.16b	

* Cultivar means at the same level of N-fertilizer, means of N fertilizer levels, and overall cultivar means followed by different letters are significantly different (DMR test) at the 5% significance level.

Only the highest nitrogen treatment significantly increased percentage of grain protein. It has been known for a long time that application of N - fertilizer after heading does not result in yield increases, but in an increase in grain protein percentage (Thorne, 1962; Neales et al., 1963). The highest nitrogen fertilizer treatment used in this experiment also increased total plant nitrogen content (Tables 5 and 6). In wheat, at the time of leaf senescence, 60-85% of the nitrogen previously stored in the leaves will translocate to the grain resulting in grain protein increases. Apparently translocation ability is different in the cultivars used in this study (Table 7). Therefore, differences in

grain protein among cultivars can be attributed to varied efficiency in absorption of nitrate, differences in translocation of reduced nitrogen from the straw to the grain and to different grain productivity of the cultivars. For example, Steptoe at the 100 + 60 nitrogen fertilizer level, yielded 7009 kg of grain (Table 1) and 863 kg of protein per hectare. Benton yielded only 5501 kg of grain but produced 799 kg of protein per hectare. There was a greater "dilution effect" in Steptoe due to its relatively higher grain production per area than with Benton which produced almost as much protein per area but had a significantly lower yeild of grain. The percentage of protein in grain in Steptoe with this treatment was 12.31% and Benton 14.50%.

I-3: Total Grain Protein Production per Hectare:

Nitrogen fertilizer treatments significantly increased total grain protein production per hectare of the four cultivars studied. At the 10% level of significance an interaction between cultivars and nitrogen levels was observed (Appendix 1). Total grain protein production increased 161% and 290% on an average for the 60 and 100 + 60 kg N/ha treatments respectively (Table 3).

Table 3. Total grain protein production, in kg/ha, of four barley cultivars under three N-fertilizer levels.

N Treatments kg/ha	Cultivars				Average
	Steptoe	Larker	Benton	Karl	
0	265a*	247a	266a	230a	252c
60	471a	398a	389a	418a	419b
100 + 60	863a	764b	799a	675b	775a

* Cultivar means at the same level of N-fertilizer, means of N levels and overall cultivar means followed by different letters are significantly different (DMR test) at the 5% level of significance.

At the 60 kg N/ha, each kg of nitrogen applied produced an average increment of 2.78 kg of grain protein per hectare. At the 100+ 60 kg N/ha treatment, the average increase in grain protein per hectare for each kg of nitrogen applied was 3.27 kg.

In regard to individual cultivars, at the 60 kg N/ha treatment, the increase in grain protein production per hectare per kg of nitrogen applied was 3.44 kg in Benton, 2.58 for Larker, 2.05 with Benton and 3.13 in Karl. In the 100 + 60 kg N/ha treatment each kg of nitrogen applied resulted in 3.78, 3.23, 3.33 and 2.78 kg of protein per hectare for the cultivars Steptoe, Larker, Benton and Karl, respectively.

The increase in grain protein production per hectare of the 60 kg N/ha treatment was due to increase in grain yield since this treatment did not increase percentage of grain protein (Tables 1 and 2). The increase observed in the 100 + 60 kg N/ha was due to increases in grain yield per hectare and in percentage of grain protein in the grain.

Differences among cultivars were also due to variation in yield and percent protein. At the 60 kg N/ha the higher grain protein production of Steptoe was due to higher grain yield (5558 kg/ha) which more than compensated for the lower protein percentage (8.49%). The cultivar Benton partially compensated its lower yield of grain (3874 kg/ha) by producing grain with a higher percentage of protein (10.03%). At the higher N-fertilizer treatment, the high total grain protein production of Steptoe (863 kg) was due to the higher grain yield of this cultivar (7009 kg/ha). The high protein production of Benton resulted from a high percentage of protein in the grain. The grain protein production of Larker and Karl were 764 and 675 kg respectively. Although Larker had a lower grain yield (5168 kg/ha), its high percentage of grain protein (14.80%) compensated for the higher grain production of Karl (5,409 kg/ha) which had only 12.50% of protein.

An interaction between cultivars and N-fertilizer on total grain protein production per area was observed. Differences in nitrogen uptake from the soil and differences in percentage of vegetative nitrogen translocated to the grain at maturity were the reasons responsible for this interaction.

I-4: Nitrate Nitrogen in Plant Tissue:

The amount of nitrate nitrogen in the plant tissue was analyzed for each cultivar, fertilizer level and at five stages of plant development. When these five stages of plant development were included in the analysis of variance, a significant interaction between stage of development and N-fertilizer level was detected (Appendix 3). Analysis of variance of each stage of development separately detected interactions

between N-fertilizer levels and cultivars at the stages of tillering, stem elongation and grain filling (Appendix 4).

Table 4 shows the average concentration of nitrate in parts per million of dry weight in the whole plant tissue of the four cultivars, three N-fertilizer levels and five stages of plant development. The average nitrate concentration of four cultivars and three N levels at the stages of tillering, stem elongation, flowering, grain filling and maturity were 2857, 474, 303, 161 and 236 parts per million of the plant dry weight respectively.

The plants of plots that received no N-fertilizer had an overall average of 101 parts per million of nitrate nitrogen accumulated in the tissue; the plants of plots that received 60 and 100 + 60 kg of N-fertilizer per hectare had respectively 589 and 1729 parts per million. At the stage of tillering the averages of four cultivars were significantly different among the three N-fertilizer levels. From stem elongation to maturity only the higher nitrogen rate produced a significantly higher concentration of nitrate in plants. An interesting observation was that nitrate nitrogen in the plant increased slightly at maturity when compared to the stage of grain filling.

Nitrate nitrogen in plant tissue reflected N-fertilizer treatments. The higher the level of nitrogen applied, the higher the concentration of nitrate in the plant. The most obvious reason for the variation in nitrate concentration in plant tissue in response to N-fertilizer levels was the availability of nitrate in the soil. Greater availability of nitrate resulted in greater uptake via active uptake and the mass action of transpiration. Welbank et al. (1974) reported that N-fertilizer greatly increased the weight of barley roots in the top 15 cm of soil.

Table 4. Nitrate-nitrogen in plant dry tissues (expressed in parts per million) of four barley cultivars under three N-fertilizer treatments and at five stages of development.

	Tillering	Stem Elongation	Flowering	Grain Filling	Maturity
0 N					
Steptoe	352 a*	58 a	70 a	52 a	53 a
Larker	150 a	49 a	53 a	42 a	84 a
Benton	353 a	58 a	67 a	45 a	70 a
Karl	226 a	59 a	37 a	53 a	74 a
Mean	270 c	56 b	56 b	50 b	70 b
60 N					
Steptoe	2978 a	91 a	56 a	61 a	67 a
Larker	1900 c	178 a	68 a	30 a	171 a
Benton	2573 bc	112 a	88 a	64 a	88 a
Karl	2793 ab	157 a	102 a	31 a	168 a
Mean	2561 b	135 b	78 b	47 b	123 b
100 + 60 N					
Steptoe	4752 b	1840 a	1014 a	860 a	678 a
Larker	5754 a	527 c	691 a	199 b	425 a
Benton	6367 a	1555 a	654 a	192 b	491 a
Karl	6082 a	1008 b	735 a	292 b	467 a
Mean	5739 a	1229 a	773 a	386 a	515 a

* Averages of cultivars at a same level of N-fertilizer and averages of N-fertilizer levels, followed by different letters are significantly different (DMR test) at the 5% level of significance.

Therefore, it is possible that nitrogen- stimulated greater root system was able to exploit a greater soil mass and consequently take up greater amounts of nutrients.

There was a decrease in nitrate concentration in the plant after tillering. This probably was due to a dilution effect resulting from the plant growing faster than its ability to take up nitrate to maintain high levels of nitrate in the tissue. An improvement in the plants' capacity to reduce nitrate through increase in nitrate reductase activity might have been another cause for the decrease in nitrate in plant tissue at later stages of plant development.

The slight increase in nitrate nitrogen at maturity suggests that nitrate is not limiting for nitrate reductase activity and nitrate content in leaves is not related with leaf senescence in barley. In other words, at senescence, the plant's ability to reduce nitrate is affected more than the plant's ability to take up nitrate from the soil.

At the 100 + 60 kg/ha N-fertilizer treatment significant differences in nitrate concentration among cultivars were observed at the stages of tillering and stem elongation. This could be explained by the differences among cultivars in nitrate uptake capacity and/or in nitrate reduction capacity.

An interaction between stages of development and N-fertilizer levels on nitrate concentration in plant tissue was observed (Appendix 3). Carpenter et al. (1952) reported that in wheat the maximum rate of nitrogen uptake from the soil is not only dependent on the stage of development, but also on the amount of nitrogen in the soil. With low nitrogen fertilizer rates, uptake usually reaches maximum around heading, but with high rates uptake may continue until soft dough stages.

It was also observed that plants from plots that received nitrogen were a few days later in maturity than the plants that received no nitrogen fertilizer. Therefore, differences in rate of nitrogen uptake at different stages of development caused by differences in nitrogen disposability and slight differences in stages of plant development caused by nitrogen fertilizer application were the causes of the interactions observed.

A-5: Percentage of Reduced Nitrogen in Plant Tissues:

Table 5 gives the percentage of reduced nitrogen in whole dry weight plant tissue of the four cultivars at three nitrogen fertilizer levels and five stages of plant development. The analysis of variance (Appendix 3) indicated a significant effect of cultivars, nitrogen fertilizer levels, and stages of plant development. A significant interaction between stage of development and fertilizer level was also observed. The average concentration of reduced nitrogen, average of four cultivars and five stages of plant development for the 0, 60 and 100 + 60 kg N/ha treatments were 1.598, 1.802 and 2.236 percent of the plant dry weight. The average of three nitrogen fertilizer levels and four cultivars, for the stages of tillering, stem elongation, flowering, grain filling and maturity were 4.228, 2.141, 1.477, 0.964 and 0.570 percent of the plant dry weight respectively. The cultivar Benton had the highest overall concentration with 1.955%, followed by Karl with 1 928, Steptoe with 1.870, and Larker with 1.712% of reduced nitrogen in plant dry weight.

Table 5. Percentage of reduced nitrogen in whole plant tissue of four barley cultivars at three N-fertilizer levels and five stages of development: tillering (1), stem elongation (2), flowering (3), grain filling (4), and maturity (5). Average of four replications.

N Treatments	Cultivars				Mean
	Steptoe	Larker	Benton	Karl	
0 N					
1	3.755 abc*	3.638 d	4.153 a	3.725 bcd	3.818
2	1.558 bc	1.488 d	1.700 ab	1.840 a	1.647
3	1.229 a	1.093 a	1.354 a	1.216 a	1.223
4	0.904 bc	0.806 c	1.054 ab	1.091 a	0.964
5	0.467 a	0.481 a	0.389 b	0.551 a	0.472
60 N					
1	4.485 a	3.878 b	4.372 a	4.373 a	4.277
2	1.950 c	1.083 bc	2.346 a	2.050 ab	2.082
3	1.344 a	1.230 a	1.469 a	1.379 a	1.356
4	0.734 bc	0.618 c	0.917 ab	0.969 a	0.810
5	0.502 a	0.444 a	0.432 a	0.565 a	.486
100 + 60 N					
1	4.592 abc	4.197 d	5.014 a	4.554 bcd	4.589
2	2.738 b	2.413 b	3.072 a	2.556 b	2.695
3	1.919 a	1.804 a	1.862 a	1.838 a	1.856
4	1.237 bc	1.112 c	1.360 ab	1.430 a	1.285
5	0.800 b	0.619 c	0.594 c	1.012 a	.756

*Mean of cultivars at the same stage of development followed by different letters are significantly different (DMR test) at the 5% probability level. At flowering stage differences are significant at the 10% probability level.

When each plant development stage was analyzed individually, interactions between cultivars and N-fertilizer levels were detected at tillering, stem elongation, and maturity. At flowering, only the effect of N-fertilizer levels was significant. The percentage of reduced nitrogen decreased from the stage of tillering to maturity (Table 5). The higher the amount of N-fertilizer applied, the higher the concentration of reduced nitrogen. At maturity Karl, a relatively low protein cultivar, had a significantly higher percentage of reduced nitrogen in the whole plant tissue than Larker and Benton, two cultivars with relatively high percentage of grain protein. Steptoe was significantly lower than Karl, but significantly higher than Larker and Benton. At maturity, plants of plots that received the highest N-fertilizer rate had an average of .756% of reduced nitrogen while plants from plots in which 0 and 60 kg N/ha were applied had an average of 0.472 and 0.486% respectively.

Like nitrate nitrogen, percentage of reduced nitrogen in dry tissue also reflected the level of N-fertilizer treatments. However, the differences among N-treatments were not as striking as in the case of nitrate nitrogen. This should be expected since at the higher N-fertilizer levels, more nitrogen was available in the soil. This greater availability of soil nitrogen also stimulated root development (Welbank et al., 1974) with a greater capacity to take up nutrients from the soil. Since reduced nitrogen in the plant is used to form structural and functional proteins (membranes, chromosomes, organelles, enzymes, storage proteins, chlorophyll, etc.) it should be expected to find differences in percentage of reduced nitrogen among plants grown in

soil with different amounts of soil-nitrogen disposible or available. In other words, plants that received larger amounts of N-fertilizer are richer in nitrogenous compounds. Differences in percentage of reduced nitrogen among cultivars can be explained by differences in rate of nitrate uptake and reduction and genetic differences in structural and functional protein among cultivars. Decreases in percentage of reduced nitrogen throughout the growing season can be accounted for by the dilution effect and the transfer of reduced nitrogen in the form of amino acids to the kernel during grain formation.

At maturity Karl, a relatively low protein producer, had a higher percentage of reduced nitrogen in the straw. Benton, a relatively high grain protein producer, had lower percentage of reduced nitrogen in the straw. This observation indicated that Karl is less efficient in the ability to translocate reduced-nitrogen to grain, whereas Benton is more efficient. Srivinas et al. (1968) studied plant protease enzyme levels in wheat cultivars having high grain protein. They observed that protease levels were higher in wheats having a high grain protein percentage. Higher amounts of proteases would break down more proteins to amino acids which are then translocated to the seeds for the synthesis of grain protein. Probably a similar mechanism also exists in barley and this would explain the differences in reduced nitrogen in the straw among cultivars observed at maturity.

Interactions between stages of development and fertilizer levels can be explained by slight differences in stages of plant development, nitrate uptake capacity at different nitrogen levels, and in capacity of nitrate reduction.

A-6: Total Reduced Nitrogen (kg/ha) on the Above Ground Vegetation at Maturity:

The total amount of nitrogen in straw and grain at maturity indicates the efficiency of plants in assimilating nitrogen from the soil. Table 6 shows the total nitrogen in straw and grain at maturity of the four barley cultivars in response to three N-fertilizer treatments.

Table 6. Total nitrogen (kg/ha) in straw and grain at maturity of four barley cultivars in response to three N-fertilizer treatments.

N Treatments kg/ha	Cultivars				Average
	Steptoe	Larker	Benton	Karl	
0	62.69a*	64.77a	57.13a	58.90a	60.87c
60	106.95a	106.80a	93.90a	108.06a	103.93b
100 + 60	211.43a	175.55a	188.60b	186.41a	190.26a
Average	127.02a	115.70a	113.13a	117.49a	

* Means of cultivars at the same N-fertilizer level, average of N-fertilizer levels and overall average of cultivars followed by different letters are significantly different (DMR test) at the 5% probability level.

No significant differences were observed in total nitrogen in above ground vegetation at maturity among the cultivars at a same level of nitrogen. Nitrogen fertilizer levels, as expected, produced significant differences. This was due to greater nitrate uptake from the soil and greater above ground vegetation development. Even though at a same level of nitrogen there was no significant difference in total above ground nitrogen produced by the four cultivars, data from Table 3 indicates

variations in the partition of plant nitrogen. Steptoe on the average transferred to the grain a greater proportion of the above ground nitrogen than Benton, Larker and Karl.

A-7: Percentage of Total Above Ground Nitrogen Retained in the Straw at Maturity:

Table 7 shows the percentage of total above ground nitrogen retained in the straw at maturity among the four cultivars and three N-fertilizer levels studied. A significant effect of cultivars as well as a significant cultivars x nitrogen levels interaction was detected (Appendix 6).

Table 7. Percentage of total above ground nitrogen (kg/ha) retained in the straw at maturity of four barley cultivars under three N-fertilizer levels.

N Treatments kg/ha	Cultivars				Average
	Steptoe	Larker	Benton	Karl	
0	32.04ab*	38.79a	25.02b	37.34a	33.30a
60	29.57b	39.57a	33.48ab	39.76a	35.60a
100 + 60	34.75ab	29.88c	32.13bc	41.10a	34.47a
Average	32.12bc	36.08ab	30.21c	39.40a	

*Means of cultivars at the same N-fertilizer level, means of N-fertilizer levels and overall means of cultivars followed by different letters are significantly different (DMR test) at the 5% probability level.

There was no significant differences among N-fertilizer levels. Among cultivars, Karl retained in the straw the highest proportion of total nitrogen per area and Benton the lowest. The cultivar Larker, at

the highest N-fertilizer, retained in the straw a significantly smaller percentage than at the low and medium levels.

Percentage of total above ground nitrogen that is retained in the straw at maturity, or conversely the proportion that is transferred to the grain, is an indication of "transport efficiency". A high transport efficiency is a desirable characteristic for high grain protein production.

A significant cultivars x N-fertilizer level interaction was detected for this trait. This means that "transport efficiency" of each cultivar varied differently according to the levels of nitrogen applied. For instance, at the low nitrogen level the cultivar Benton transferred to the grain a greater percentage of the above ground nitrogen than at the medium and high levels. Larker was just the opposite: at the high N-fertilizer, it transferred to the grain a higher proportion of the total nitrogen in the above ground vegetation. The cultivars Steptoe and Karl did not vary in "transport efficiency" at different levels of nitrogen fertilizer applied.

Differences in proteases inducibility can be postulated as the factors responsible for the variation in "transport efficiency" observed.

I-8: Effect of Nitrogen Fertilizer Levels upon Nitrate Reductase Activity:

Table 8 summarizes the nitrate reductase activity, expressed as moles of NO_3 reduced per gram of leaf weight per hour, of the four cultivars and three N-fertilizer levels. In Appendix 8 the ANOVA table is shown. Nitrate reductase activity (NRA) was significantly affected by N-fertilizer levels, cultivars and stages of plant development.

Table 8. Nitrate Reductase Activity (μ moles of NO_3 reduced per gram of leaf fresh weight per hour) of four barley cultivars at five stages of plant development and three N-fertilizer levels. Average of four replications.

	Tillering	Stem Elongation	Flowering	Grain Filling	Maturity	Average
0 N						
Steptoe	0.943 bc*	0.654 a	1.244 a	3.523 bc	--	1.273
Larker	0.812 d	0.517 bc	1.462 a	3.674 b	--	1.293
Benton	1.180 a	0.364 c	0.684 b	3.298 c	--	1.105
Karl	0.992 ab	0.621 ab	1.435 a	4.119 a	--	1.433
Average	0.982	0.539	1.206	3.653		1.276
60 N						
Steptoe	1.131 a	0.923 a	1.172 abc	3.502 a	--	1.346
Larker	1.145 a	0.876 bc	1.067 bc	3.658 a	--	1.349
Benton	1.397 a	0.778 c	0.805 c	3.620 a	--	1.320
Karl	1.160 a	0.909 ab	1.489 a	3.677 a	--	1.447
Average	1.208	0.872	1.133	3.614		1.365
100 + 60 N						
Steptoe	1.175 a	1.319 a	2.395 a	4.192 bc	--	1.816
Larker	1.149 a	1.032 bc	1.957 bc	4.415 a	--	1.711
Benton	1.293 a	1.047 c	1.290 c	3.898 c	--	1.507
Karl	1.161 a	1.135 ab	2.210 ab	4.439 a	--	1.789
Average	1.194	1.133	1.963	4.211		1.700

* Means at a same stage of plant development followed by different letters are significantly different (DMR test) at the 5% level of probability. At tillering and grain filling, differences are significant at the 10% probability level.

No interactions between stages of development and cultivars or stages of development and nitrogen levels were observed. However, when each stage of development was analyzed separately, interactions between cultivars and N-fertilizer levels were detected at tillering, flowering and grain filling (Appendix 7).

After tillering a decline in NRA was observed until the stage of stem elongation followed by an increase in enzyme activity that culminated at the stage of grain filling (Table 9).

Table 9. Average NRA (μ moles of NO_3 reduced per gram of leaf fresh weight per hour) of four barley cultivars at five stages of plant development.

Stages of Plant Development				
Tillering	Stem Elongation	Flowering	Grain Filling	Maturity
1.119 cd*	0.848 d	1.434 bc	3.826 a	--

* Means followed by different letters are significantly different (DMR test) at the 5% probability level.

The decline in enzyme activity after tillering was more accentuated in the plots that received no nitrogen (Table 8).

Table 10 shows the average NRA of the four cultivars throughout the stages in which NRA was detected. Karl had the highest overall average enzyme activity whereas Benton had the lowest average NRA. Steptoe and Larker were intermediate.

Table 10. Average NRA (μ moles of NO_3 reduced per gram of leaf fresh weight per hour) of four barley cultivars throughout four stages of plant development and three N-fertilizer levels.

Cultivar			
Karl	Steptoe	Larker	Benton
1.937 a*	1.848 abc	1.813 bc	1.541 c

* Means followed by different letters are significantly different (DMR test) at the 5% probability level.

Nitrate reductase activity was also affected by levels of nitrogen fertilizer. Table 11 indicates the average enzyme activity for each nitrogen fertilizer level.

Table 11. Average of four cultivars and four stages of plant development NRA (μ moles of NO_3 reduced per gram of leaf fresh weight per hour) of three N-fertilizer levels.

Nitrogen Fertilizer Levels (kg/ha)		
0	60	100 + 60
2.126 a*	2.278 b	2.834 a

* Means followed by different letters are significantly different (DMR test) at the 5% probability level.

At the highest nitrogen rate, average enzyme activity increased 33% when compared with the activity observed at the 0 N-fertilizer treatment. The intermediate N-fertilizer level increased average enzyme activity by 7%.

Nitrate reductase activity was affected by cultivars, N-fertilizer rates and stages of plant development. The increase in activity in response to nitrogen levels was expected since nitrate reductase is an inducible enzyme (Ingle et al., 1966).

It is interesting to observe that this NRA increase in response to N-fertilizer was much more remarkable at the stage of stem elongation. At that stage enzyme activity increased an average of 61 and 110% in the 60 and 100 + 60 kg N/ha respectively.

The stem elongation stage had the lowest NRA. This may be attributed to the lag of nitrate reductase synthesis behind leaf weight increase at this very rapid growth stage.

Nitrate reductase activity is regulated not only by the amount of nitrate in the short-lived inducing pool (Heimer and Filner, 1971), but also by other factors such as end-product repression (Kinsley, 1961), amino-acids pool size (Filner, 1966), plant hormones (Lips and Roth-Benjerano, 1969) and environmental variables such as availability of nutrients, light intensity, ambient temperature, etc (Beevers and Hageman, 1969; Kessler, 1964). Therefore, the variation in NRA in the four cultivars in response to N-fertilizer.

Differences in enzyme activity among cultivars was due to differential inducibility characteristics of each cultivar (Zeiserl et al., 1963). The increase in NRA observed after stem elongation probably reflects an increased need of reduced nitrogen for DNA, RNA, and protein for pollination, fertilization and grain development. This increment can be due to de novo synthesis for nitrate reductase has a short half-life (Hageman and Flesher, 1960) or reactivation of previously inactive forms.

Interactions between cultivars and N-fertilizer levels can be explained by differences in enzyme inducibility. For example, the cultivar Benton at the stage of flowering, increased NRA by 18 and 88% in response to 60 and 100 + 60 kg N/ha respectively when compared with the 0 nitrogen treatment. On the other hand, in the cultivar Karl the corresponding increments in NRA were 4 and 54% respectively. Similar variation was observed for the other cultivars and stages of development.

I-9: Daily Input of Reduced Nitrogen Based on Nitrate Reductase Activity Assay:

Daily input of reduced nitrogen is the theoretical daily input of reduced nitrogen based upon the level of enzyme activity and total leaf weight of above ground vegetation per hectare. The assumption is made that nothing is limiting nitrate reduction and that all above ground vegetation has the same nitrate reductase activity as the leaves that were sampled. Since these assumptions are not fulfilled, it is an overestimation of the actual daily input of reduced nitrogen. However, it is useful for it considers differences in growth rate and weight of above ground vegetation that exists among cultivars. Table 12 shows the daily input of reduced nitrogen based on NRA of the four barley cultivars studied.

The theoretical daily input of reduced nitrogen increased with level of N-fertilizer and stage of development. At tillering, a significant (10% probability level) effect of fertilizer was detected but neither effect of cultivars or interaction was observed. At the stage of stem elongation both the effects of N-fertilizer levels and cultivars were significant (Appendix 8). A similar observation was noted at flowering. During grain filling only the effect of nitrogen levels was significant.

Table 12. Daily input of reduced nitrogen based on NRA in grams per hectare per day, of four barley cultivars at four stages of plant development and three N-fertilizer levels.

	Tillering	Stem Elongation	Flowering	Grain Filling	Average
0 kg H/ha					
Steptoe	.443 a	1.241 a*	2.972 a	11.184 a	3.960
Larker	600 a	1.069 a	4.337 a	11.252 a	4.315
Benton	695 a	655 a	1.486 b	10.716 a	3.388
Karl	484 a	949 a	3.085 a	10.575 a	3.776
Average	558	979	2.970	10.932	3.860
60 kg N/ha					
Steptoe	1.603 a	3.593 b	5.061 a	18.342 a	7.150
Larker	1.447 a	5.152 a	7.179 a	21.556 a	8.834
Benton	1.628 a	2.878 b	4.000 b	19.957 a	7.116
Karl	1.518 a	3.540 b	7.009 a	20.825 a	8.223
Average	1.549	3.791	5.812	19.845	7.831
100 + 60 kg H/ha					
Steptoe	1.487 b	6.962 a	16.188 a	27.914 a	13.138
Larker	2.136 a	6.342 ab	14.113 a	28.346 a	12.734
Benton	1.411 b	4.998 c	8.144 b	22.307 b	9.215
Karl	1.610 b	5.248 bc	14.665 a	28.571 a	12.524
Average	1.661	5.888	13.277	26.785	11.903

* Means of cultivars at the same level of N-fertilizer followed by different letters are significantly different (DMR test) at the 5% significance level. At tillering differences are significant at the 10% level of significance.

The cultivar Benton had the lowest overall average daily input of reduced nitrogen based on NRA assay. Larker had the highest. The 60 kg H/ha treatment increased daily input of reduced nitrogen by an average of 102%. The 100 + 60 kg N/ha treatment produced a 208% average increase in theoretical daily input of reduced nitrogen when compared to plots that received no nitrogen.

The increment observed with N-fertilizer was expected since it increased plant growth and nitrate reductase activity (Table 6, 8 and 11). The increase exhibited at successive stages of plant development was due to increased plant fresh weight per area and increase in NRA. At the stage of stem elongation a decrease in NRA occurred. However, this was more than compensated for by an increase in plant fresh weight per hectare. As a result, daily input of reduced nitrogen based on enzyme assay increased when compared to the tillering stage.

Differences among cultivars were due to variations in NRA and above ground vegetation fresh weight. At tillering Benton had a high daily input due to high NRA, but at later stages the daily input of reduced nitrogen was less than the other cultivars because Benton had a relatively low NRA after that stage.

I-10: Theoretical Input of Reduced Nitrogen Throughout the Growing Season:

Table 13 summarizes the theoretical and actual input of reduced nitrogen per hectare throughout the entire growing season.

Table 13. Theoretical and actual seasonal input of reduced nitrogen, in kg per hectare, in the above ground vegetation of four barley cultivars at three N-fertilizer levels.

	Theoretical Input	Actual Input	Ratio ("Enzyme efficiency")
Treatment: 0 kg N/ha			
Steptoe	248.19	62.69	3.96
Larker	268.74	64.77	4.15
Benton	218.29	57.13	3.82
Karl	237.15	38.90	4.03
Average	243.09	60.87	3.99
Treatment: 60 kg N/ha			
Steptoe	445.34	106.95	4.16
Larker	542.63	104.84	5.18
Benton	419.09	93.90	4.46
Karl	510.31	110.25	4.63
Average	479.34	103.99	4.61
Treatment: 100 + 60 kg N/ha			
Steptoe	791.66	211.43	3.74
Larker	775.99	175.55	4.42
Benton	565.50	188.47	3.00
Karl	763.77	186.41	4.10
Average	724.23	190.47	3.80

The ratio between the two values is an indication of "enzyme efficiency", that is, total nitrate reductase activity per unit of reduced nitrogen accumulated. The lower the ratio, the higher the "enzyme efficiency". Although the ratios obtained varied between 3.00 and 5.18, the correlation between theoretical and actual accumulation of reduced nitrogen (Table 13) was significant ($R = 0.94$).

The cultivars Benton and Steptoe showed a higher "enzyme efficiency" than Karl and Larker. At the 60 kg N/ha treatment, the efficiency of Steptoe was higher than Benton; otherwise, the ranking of the cultivars was the same for all three N-fertilizer treatments. The average ratios for the zero, medium and high N-fertilizer levels were 3.99, 4.61 and 3.82 respectively.

The 3.00 to 5.13 ratio between theoretical and actual seasonal input of reduced nitrogen could be due to several causes. The most important was the necessary assumption that all leaves and stems had the same NRA as the flag leaves that were sampled. This certainly was not the case for it is known that leaves decrease in NRA with age and mutual shading and stems have a lower enzyme activity than leaves. It was also assumed that nitrate reduction occurs at a constant rate during 12 hours per day. This probably does not occur under field conditions where temperature most of the time is either below or above the optimum for maximum nitrate reduction. Intermittent feedback inhibition of enzyme activity was also a possibility. It is known that roots also have some nitrate reductase activity which was not measured in this experiment. Finally, there is the possibility that enzyme activity as determined in the laboratory does not reflect exactly the <u>in situ</u> activity in the plant.

Klepper (1975) considered the in vivo method for NRA assay as used in this experiment as superior to the in vitro method for the reason that it better imitates the in situ conditions in the plant.

It is interesting to observe that the cultivar Benton had a lower seasonal average NRA and a higher "enzyme efficiency" which partially compensates for it. The total nitrogen in straw and grain at maturity of Benton (Table 6) was not significantly different from other cultivars with higher NRA, but slightly lower "enzyme efficiency". To calculate theoretical and actual input of reduced nitrogen, a large number of samples and laboratory analyses must be conducted (plant fresh and dry weight/area, total nitrogen, nitrate nitrogen, NRA) as well as considerable number of calculations. Each of these operations is liable to sampling and technique errors that eventually might bias the results. The good correlation between the calculated and actual values obtained in this experiment suggests that those errors were within an acceptable limit or that there was some compensation among the values.

The observation that the ranking in "enzyme efficiency" can be altered by level of nitrogen fertilizer suggests that this trait is not only affected by cultivars, but by environmental conditions as well.

I-11: Glutamine Synthetase Activity:

Glutamine synthetase is another important enzyme in the nitrogen assimilation pathway. It not only plays a key role in the assimilation, storage and translocation of ammonia, but glutamine itself is the substrate for the synthesis of numerous plant metabolites.

This enzyme was analyzed at the soft dough stage. A comparison was made between cultivars that received 100 + 60 kg N/ha and the

cultivar Benton was assayed at three N-fertilizer levels.

Table 14 shows the glutamine synthetase activity of the four cultivars studied.

Table 14. Glutamine synthetase activity (μ moles of glutamyl hydroximate produced in ten minutes per gram of leaf fresh weight) of four barley cultivars under 100 + 60 kg N/ha fertilizer treatment.

Cultivar			
Steptoe	Larker	Benton	Karl
0.9700 bc*	0.6467 c	1.0467 abc	1.2067 a

* Means followed by different letters are statistically different (DMR test) at the 5% probability level.

Karl had the highest glutamine synthetase activity and Larker the lowest.

Glutamine synthetase activity was also expressed as specific activity, that is, the enzyme activity based on the amount of extracted soluble protein in leaf tissue. Table 15 indicates the specific glutamine synthetase activity of the four cultivars.

Table 15. Specific activity of glutamine synthetase (μ moles of glutamyl hydroxymate produced per minute per mg of soluble protein in leaf tissue) of four barley cultivars under 100 + 60 kg N/ha treatment.

Cultivar			
Steptoe	Larker	Benton	Karl
1.058 a*	0.7988 b	0.8996 b	1.1704 a

* Means followed by different letters are significantly different (DMR test) at the 5% level of significance.

The cultivars Steptoe and Karl had significantly higher specific activity than either Larker or Benton.

Table 16 presents the specific glutamine synthetase activity of the cultivar Benton at three nitrogen fertilizer levels.

Table 16. Specific activity of glutamine synthetase (μ moles of glutamyl hydroxymate produced per minute per mg of soluble protein in leaf tissue) of the cultivar Benton at three N-fertilizer levels.

Nitrogen Fertilizer (kg/ha)		
0	60	100 + 60
1.4109a*	1.0164b	0.9611b

* Means followed by different letters are significantly different (DMR test) at the 5% level of significance.

At the zero nitrogen treatment, the specific activity of glutamine synthetase was significantly higher than at the 60 and 100 + 60 kg N/ha treatments.

Examination of Tables 1, 3, 14, 15, and 16 shows no apparent relationship between glutamine synthetase activity and the agronomic performance of the four cultivars studied. Glutamine synthetase is considered one of the main ports of entry of ammonia into amino acids. However, another enzyme, glutamate dehydrogenase, plays a similar role though by a different pathway. It is possible that both systems function simultaneously in leaves; therefore, the lack of association observed. Glutamine does not only serve as a storage compound for ammonia which later will be transferred to keto-acids, but it is also the base of many compounds formed by the plant cell such as nucleotides.

This also could be responsible for the apparent lack of association between glutamine synthetase acitivity and grain yield and grain protein production. Specific activity of glutamine synthetase of the cultivar Benton decreased with increasing N-fertilizer (Table 16). This reduction in specific activity was due to a disproportional increase in different enzyme proteins by N-fertilizer application. Since enzyme specific activity is the expression of activity in relation to the amount of soluble protein in leaf extract, greater amounts of protein will result in reduced specific activity.

I-12: Nitrate Reductase Activity and its relationship to Nitrogen-Yield Criteria.

Nitrogen-yield criteria is here defined as the amount of nitrate-nitrogen and reduced nitrogen in the tissue, total reduced-nitrogen per area and yield of grain and grain protein production. Nitrate reductase activity contributes to N-yield criteria since it is the enzyme responsible for the initial reduction of nitrate in the plant.

A regression analysis was conducted by regressing several N-yield criteria on NRA or / and daily input of reduced nitrogen based on NRA assay. The model of regression analysis adopted was the quadratic model. Both linear and quadratic models were evaluated. From the regression analysis a coefficient of multiple determination (R^2) was obtained, which is a descriptive measure of the degree of relation between the dependent and independent variables. It gives the proportionate reduction of the total variation in Y (dependent variable) that can be explained by regressing Y on X (independent variable) according to the model used. In this study, NRA and daily input of reduced nitrogen based on NRA were considered as the independent variables except in the relationship between NRA and nitrate nitrogen where nitrate nitrogen was the independent variable.

I-13: Nitrate Reductase Activity and Nitrate Nitrogen in Plant Tissue:

Nitrate reductase is a substrate inducible enzyme. Therefore, an association between the enzyme activity and the amount of nitrate nitrogen in plant tissue should be expected. Table 17 exhibits the coefficients of multiple determination (R^2) values between nitrate nitrogen in whole plant tissue and NRA, average of four cultivars and at the stages of development at which NRA was assayed.

Table 17. Coefficients of multiple determination (R^2) between nitrate nitrogen in plant tissue and NRA. Average of four barley cultivars and at five stages of plant development.

	Stage of Plant Development				
	Tillering	Stem Elongation	Flowering	Grain Filling	Maturity
R^2 Values	0.16	0.49	0.38	0.02	0.00

At the stage of stem elongation, by regressing NRA on plant nitrate-nitrogen, 49% of the variation in NRA can be explained by the regression. At maturity no association was observed because no enzyme activity was detected at this stage. At grain filling the association was very low.

The highest R^2 values were observed at the stages of stem elongation and flowering--0.49 and 0.38 respectively. However, a perfect association between nitrate plant tissue and NRA is not necessary. Heimer and Filner (1971) studied the nitrate assimilation pathway in cultured tobacco cells. From their observations they concluded that most of the nitrate which the cell accumulates is not necessary for induction, nor does it affect induction rate. Most of the nitrate is available as substrate for nitrate reductase, but very little of it can

perform inducing function of nitrate. In other words, there are two pools or compartments of nitrate in the cells--a small short-lived inducing pool and a large, long-lived substrate pool. Nitrate in the substrate pool cannot replenish the inducing pool.

This could explain the variation observed in the R^2 values at the different stages of plant development. That is, the relative sizes of the substrate and inducing pools vary with plant growth and development. At the stage of stem elongation, a greater proportion of the total plant nitrate content would be located in the inducing pool of the cell, therefore, the higher degree of association observed.

I-14: Nitrate Reductase Activity and Percentage of Reduced Nitrogen in Plant Tissue:

In Table 18 are the R^2 values between nitrate reductase activity and percentage of reduced nitrogen in plants at several stages of plant development.

Table 18. R^2 values between NRA and percentage of reduced nitrogen in plant tissue. Average of four cultivars and at five stages of plant development.

	Stage of Plant Development				
	Tillering	Stem Elongation	Flowering	Grain Filling	Maturity
R^2 Values	0.34	0.52	0.16	0.12	0.00

The R^2 values between NRA and percentage of reduced nitrogen in plant tissue varied according to the stage of plant development. Again the highest value was detected during stem elongation. At tillering the

variation between increment in enzyme activity and increment in percentage of reduced nitrogen was out of proportion in relation to one another, as a result of N-fertilizer application (Tables 5 and 8). This lack of proportionality or correlation was more accentuated in the cultivars Larker and Benton. For example, in Larker the 60 and 100 60 kg N/ha increased NRA by 41 and 42% respectively, but percentage of reduced nitrogen in the tissue increased .24 and .56% respectively. Benton increased average enzyme activity by 18 and 9%, but percentage of reduced nitrogen in the tissue increased .22 and .86% respectively. In other words, the variation between NRA and percentage of reduced nitrogen was less at the stage of stem elongation, therefore the higher R^2.

I-15: Daily Input of Reduced Nitrogen Based on NRA Assay and Total Reduced Nitrogen (kg/ha) in the Above Ground Vegetation:

Daily input of reduced nitrogen from enzyme assay also considers differences in vegetative growth among cultivars. Therefore a good relationship should be expected between the daily input of reduced nitrogen and total reduced nitrogen (kg/ha). Table 19 gives the coefficients of multiple determination between daily input of reduced nitrogen based on NRA and total reduced nitrogen in the above ground vegetation on a per hectare basis.

Table 19. R^2 values between daily input of reduced nitrogen from NRA assay and total reduced nitrogen in above ground vegetation per hectare. Average of four barley cultivars and at five stages of development.

	Stages of Plant Development				
	Tillering	Stem Elongation	Flowering	Grain Filling	Maturity
R^2 Values	0.73	0.85	0.75	0.46	0.00

At stem elongation 85% of the variation in total reduced nitrogen per hectare can be explained by the regression. The lowest R^2 was observed at the stage of grain filling. The values obtained were high considering that all four cultivars were included together when regressing daily input of reduced nitrogen on total reduced nitrogen per area. In those cases, differences in "enzyme efficiency" among cultivars tend to lower R^2 values somewhat because it is one more variable introduced that the regression cannot explain.

Nitrate reductase activity, one of the components of the daily input of reduced nitrogen, showed a good relationship with percent of reduced nitrogen at stem elongation (Table 18). This should explain the better association between daily input of reduced-nitrogen based on enzyme assay and total reduced-nitrogen/area observed at stem elongation. However, the same rationale does not explain the relatively high R^2 detected at tillering and flowering.

I-16: Nitrate Reductase Activity and Percentage of Grain Protein at Maturity:

Table 20 shows the proportion of the variation in percentage of grain protein at maturity that can be accounted for by regressing percent of grain protein of eqch individual cultivar on the corresponding NRA.

The highest coefficients of multiple determination were observed at the stage of flowering with an average R^2 of 0.60. However, when all four cultivars were analyzed together, the coefficient of multiple determination at flowering decreased to 0.20.

Table 20. R^2 values between NRA and percentage of grain protein at maturity of four barley cultivars.

Cultivar	Stages of Plant Development			
	Tillering	Stem Elongation	Flowering	Grain Filling
Steptoe	0.05	0.51	0.57	0.12
Larker	0.02	0.20	0.46	0.42
Benton	0.00	0.42	0.72	0.09
Karl	0.09	0.50	0.65	0.07
Average	0.04	0.41	0.57	0.18

Within each cultivar, the variation in percentage of grain protein appears to be highly associated with nitrate reductase activity at the stage of flowering. It is difficult to explain the reason for the higher R^2 values detected at flowering. One possible reason would be that at the stage of flowering, most of the reduced nitrogen in the flag leaf is translocated to the grain for the synthesis of protein. It is possible that the nitrogen reduced before flowering is used for vegetative growth which reaches the maximum at the onset of flowering. This is the photosynthetic area that synthetizes carbohydrates to be later translocated to the grain. The nitrogen reduced after onset of flowering is no longer used for leaf area formation, but for the formation of enzymes and proteins in the leaves. These proteins are later broken down and the amino acids translocated to the grain for protein formation. The greater the NRA at this stage, the greater the amount of protein formed and translocated to the grain. Therefore, the higher association observed at this stage of plant development.

When all cultivars were considered together the R^2 between NRA and percentage of grain protein at maturity decreased drastically to 0.20. This would be expected since the cultivars differed in regard to enzyme inducibility, "enzyme efficiency" and "translocation efficiency". This suggests that NRA alone as a selection criterion for percentage of grain protein is of limited value since other factors, besides NRA, might affect percentage of grain protein among different cultivars.

I-17: Nitrate Reductase Activity and Total Grain Protein Production per Hectare:

To eliminate the effects due to cultivar differences, total grain protein production per hectare of each cultivar was individually regressed on the corresponding nitrate reductase activity.

Table 21 gives the R^2 values obtained for each cultivar and stage of development.

Table 21. R^2 values between NRA and total grain protein production per hectare of four barley cultivars at four stages of plant development.

Cultivar	Stages of Plant Development				Cultivar Total
	Tillering	Stem Elongation	Flowering	Grain Filling	
Steptoe	0.08	0.66	0.72	0.30	1.76
Larker	0.03	0.37	0.51	0.40	1.31
Benton	0.01	0.60	0.79	0.65	2.05
Karl	0.29	0.54	0.55	0.12	1.50
Average	.10	.54	0.64	0.37	

The greatest R^2 values obtained were detected at the stage of flowering. However, when all four cultivars were lumped together and a regression analysis was conducted across all four cultivars, the coefficients of multiple determination for the stages of stem elongation and flowering were 0.50 and 0.41 respectively.

Total grain protein production is dependent on grain yield and percent of grain protein. Since NRA is related to grain yield and percentage of grain protein within a same cultivar, a good relationship between enzyme activity and total grain protein production should be expected even among cultivars. The high R^2 obtained confirms that NRA is a good total grain protein predictor.

I-18: Daily Input of Reduced Nitrogen Based on NRA Assay and Total Grain Protein Production per Hectare:

Daily input of reduced nitrogen based on NRA assay should be more closely associated with total grain protein per area for it takes into consideration differences in vegetative mass among cultivars. It is a relative measure of NRA per area rather than NRA per gram of leaf fresh weight. Since the highest R^2 values between NRA and total grain protein were observed at flowering, Table 22 gives the coefficients of multiple determination for each cultivar at that stage.

Table 22. R^2 values between daily input of reduced nitrogen based on NRA and total grain protein per hectare of four barley cultivars at the stage of flowering.

	Cultivars			
	Steptoe	Larker	Benton	Karl
R^2 Values	0.87	0.68	0.72	0.75

When the four cultivars were considered together and a quadratic regression analysis of daily input of reduced nitrogen on total grain protein per hectare was computed, the R^2 at flowering was 0.65.

Higher R^2 values were obtained than by regressing total grain protein per area on NRA. The high R^2 (0.65) obtained was remarkable when considering all four cultivars together. This might best be explained by the fact that vegetative leaf area is favorably associated with yield. Leaf area is also used in calculating daily input of reducted nitrogen; therefore, a higher association with yield and protein content should be expected than with NRA alone. Nitrate reductase activity at flowering was closely associated with percent grain protein within the same cultivar (Tables 20 and 21). This suggests that differences in "enzyme efficiency" and "transport efficiency" do not have the overwhelming effect as it first appears when making comparisons among cultivars. Rather, it is the capacity of the whole plant canopy for a given area to reduce a certain amount of nitrogen per day. At least part of the variation of the R^2 value 0.35 not explained by the regression would then be due to differences in "enzyme efficiency" and "transport efficiency."

I-19: Nitrate Reductase Activity and Grain Yield per Hectare:

Yield of grain is the single most important attribute of cereals. Therefore, the relationship between NRA and yield of grain was assessed by regressing enzyme activity on yield. The R^2 values for each cultivar and stage of plant development are presented in Table 23.

Table 23. R^2 values between NRA and grain yield per hectare of four barley cultivars and at four stages of plant development.

Cultivars	Stages of Plant Development				Seasonal Average NRA
	Tillering	Stem Elongation	Flowering	Grain Filling	
Steptoe	0.05	0.62	0.59	0.30	0.69
Larker	0.14	0.59	0.41	0.29	0.52
Benton	0.08	0.75	0.81	0.15	0.53
Karl	0.40	0.43	0.32	0.14	0.32
Average	0.17	0.60	0.53	0.22	0.52

The highest R^2 values were detected at stem elongation and flowering. The seasonal average for NRA also showed a good association with grain yield, but in general lower than at the two stages cited above.

If a breeding criterion is to be of value in improving crops, it must be valid when tested among genotypes. Therefore, the effects due to cultivar differences were considered for all four genotypes combined. Table 24 shows the R^2 values between NRA and yield when averaging cultivars and the four stages of development.

Table 24. R^2 values between NRA and yield of grain per hectare. Average of four cultivars and at four stages of plant development.

	Stages of Plant Development			
	Tillering	Stem Elongation	Flowering	Grain Filling
R^2 Values	0.04	0.55	0.37	0.09

Yield of grain per hectare when regressed on seasonal average NRA--that is, the average enzyme activity of all stages of plant development--resulted in an R^2 of 0.36. The R^2 for seasonal average and individual cultivars was 0.60 for Steptoe, 0.52 for Larker, 0.53 for Benton and 0.32 for Karl.

In this experiment the highest degree of association between NRA and grain yield was observed at stem elongation. Therefore the equation:

$$Y = 1{,}555.69 + 345\ X\ -\ 119.50\ X^2$$

would have predicted the average yield performance of the four cultivars under the conditions of this experiment. The average was used because plant breeders using NRA as a criterion for selection for yield would be interested in an average based on a large number of cultivars and, if possible, averages of several years. Like heritability estimates, averages are more important than values obtained for one cultivar and for one year.

The greatest degree of association was observed at stem elongation. One possible hypothesis to explain this would be that NRA at this stage is related to the potential of the total leaf area which is in turn related to the yield potential of the plant.

I-20: Coefficients of Multiple Determination Between Daily Input of Reduced Nitrogen Based on NRA Assay and Grain Yield:

R^2 values obtained by regressing daily input of reduced nitrogen are presented in Table 25.

Table 25. R^2 values between daily input of reduced nitrogen from enzyme assay and grain yield per hectare across four barley cultivars and at four stages of development.

	Stages of Development			
	Tillering	Stem Elongation	Flowering	Grain Filling
R^2 Values	0.35	0.76	0.55	0.47

There was a considerable increase in R^2 values when yield was regressed on daily input of reduced nitrogen comapred with the R^2 values obtained when yield was regressed on NRA.

Hageman et al. (1975) stated that "biochemical criterion should show a reasonable relationship to productivity or quality of the product." They did not define how much can be considered as "reasonable". Fisher (1975) discussing the future role of physiology in wheat breeding, staged that he believed significant correlation values even as low as $R = 0.30$ are useful, especially as it is possible to combine a whole series of such yield-related characters into selection indices.

It is very difficult to satisfactorily test the importance of a given selection criterion because there is always the possibility of a confounding effect exerted by differences in genetic background. In the case of NRA, differences in leaf area, "enzyme

efficiency" and "transport efficiency" play an important role. The results suggest that daily input of reduced nitrogen based on enzyme assay is a better yield predictor than NRA alone, for it considers NRA per area and not per unit of leaf weight.

I-21: Simultaneous Contribution of NRA, "Enzyme Efficiency", and "Transport Efficiency" to Yield of Grain and Total Grain Protein Production per Hectare:

A stepwise multiple regression analysis was conducted to study the contributions of NRA, "enzyme efficiency" and "transport efficiency" at stem elongation to grain yield and grain protein production.

"Enzyme efficiency" (EE) and "transport efficiency" (TE) were calculated for each of the 48 entires. Table 26 shows the results observed.

Table 26. Summary of stepwise multiple regression analysis of NRA, EE, and TE in yield of barley.

Entered Variable	R^2	Residual Mean Squares
NRA	.5075	1,290,925
EE	.5227	1,278,971
$(EE)^2$	.5276	1,294,645
TE	.5287	1,321,635
$(NRA)^2$	.5291	1,351,853
$(TE)^2$	.5291	1,384,825

The contribution of enzyme efficiency was relatively small to the total R^2. The value of "transport efficiency" to the final R^2 was also minimized.

$Y = 1458 + 3350$ NRA predicts average yield of grain.

Table 27 shows the contribution of NRA, EE, and TE to total grain protein per hectare.

Table 27. Summary of stepwise multiple regression analysis of NRA at stem elongation, "enzyme efficiency" and "transport efficiency" in total grain protein production.

Entered Variable	R^2	Residual Mean Squares
NRA	.4957	28.711
EE	.5013	29.023
$(EE)^2$	.5036	29.545
$(TE)^2$	.5071	30.015
TE	.5085	30.643
$(NRA)^2$	.5086	31.386

The equation:

$$Y = 68 + 488\ NRA$$

would have predicted average total grain protein production per hectare.

I-22: Simultaneous Contribution of Daily Input of Reduced Nitrogen Based on NRA (DIRN), "Enzyme Efficiency" and "Transport Efficiency" at Stem Elongation to Yield of Grain and Grain Protein Production per Hectare:

Table 28 summarizes the contribution of daily input of reduced nitrogen at stem elongation, "enzyme efficiency" and "transport efficiency" to grain yield.

Table 28. Summary of contribution of daily input of reduced nitrogen at stem elongation, "enzyme efficiency", and "transport efficiency" to grain yield.

Variable Entered	R^2	Residual Mean Squares
DIRN	.6808	836.742
EE	.7104	775.972
$(DIRN)^2$	.7156	779.410
$(TE)^2$	.7217	780.317
$(EE)^2$	.7259	786.969
TE	.7273	801.915

The equation:

$$Y = 3272 + 555 \text{ DIRN} - 219 \text{ EE}$$

would have predicted average yield performance. "Enzyme efficiency" has a negative effect because it is inversely proportional to the ratio between predicted and actual input of reduced nitrogen throughout the growing season. In other words, the greater the value the lower the "enzyme efficiency".

Table 29. Summary of stepwise multiple regression analysis of daily input of reduced nitrogen (DIRN) at stem elongation, "enzyme efficiency" and "transport efficiency" in total grain protein per hectare.

Entered Variable	R^2	Residual Mean Squares
DIRN	.7448	14.527
EE	.7948	11.940
$(TE)^2$	.8054	11.580
TE	.8140	11.328
$(DIRN)^2$	.8159	11.479
$(EE)^2$	.8176	11.650

The equation:

$$Y = 357 + 86\ DIRN - 42\ EE$$

predicts average grain protein production per hectare.

R^2 values above .99 were obtained when regressing average daily input of reduced nitrogen, "enzyme efficiency" and "transport efficiency on the average of four replications for total grain protein production The corresponding value when all 48 entries were considered separately was .82 (Table 29). This lower value observed when individual observations were regressed was due to the fact that the regression cannot explain the variation of individual observations around the mean of four replications.

It is an interesting observation that daily input of reduced nitrogen based on NRA assay again was a better yield and total grain protein predictor than NRA alone. "Enzyme efficiency" significantly increased R^2 values when daily input of reduced nitrogen was used as a predictor of yield and grain protein production; however, it did not increase R^2 significantly when NRA was regressed. "Transport efficiency" did not significantly increase the R^2 when 48 entries were regressed on yield and grain protein production per area. It is difficult to explain the reason for the apparent lack of effect of "transport efficiency" on total grain protein per area.

II-1: Nitrate Reductase Activity as Related to Yield and Protein in Field Grown F_2 Segregating Plants:

In F_2 segregating individual plants from crosses of some of the parents used in Experiment I, NRA was measured and regressed on yield and grain protein production. NRA was assayed at the stage of grain

filling only. Table 30 shows the coefficients of multiple determination obtained.

Table 30. Coefficients of multiple determination between NRA at the grain filling period and grain yield, percent protein in grain and total grain protein per plant of individual F_2 plants grown in field.

Cross	Yield	Percent Protein in Grain	Total Grain Protein /Plant
Steptoe x Karl	0.08	0.15	0.27
Benton x Steptoe	0.40	0.06	0.51
Benton x Larker	0.24	0.57	0.35
Benton x Karl	0.00	0.03	0.09
Larker x Steptoe	0.24	0.14	0.12

High and low R^2 values were observed as well as considerable variability. One of the probable causes of the variation observed was the fact that NRA was assayed only at grain filling. At the sampling time it was not known that better association would have been obtained at earlier stages of growth. The fact that plants were solid seeded may have resulted in a confounding effect due to competition.

III-1: Nitrate Reductase Activity as Related to Yield and Protein in F_2 Segregating Plants Grown in the Greenhouse.

F_2 plants were grown in the greenhouse and NRA measured at the grain filling period. Table 31 shows the R^2 values obtained by regressing NRA on yield and grain protein.

Table 31. Coefficients of multiple determination between NRA during grain filling and yield. Percent of grain protein and total protein per plant of F_2 plants grown in greenhouse.

Cross	Yield	Percent Protein in Grain	Total Grain Protein /Plant
Larker x Karl	0.12	0.11	0.08
Karl x Steptoe	0.07	0.31	0.17
Larker x Steptoe	0.47	0.27	0.37
Steptoe x Benton	0.14	0.01	0.37
Karl x Benton	0.57	0.21	0.66

As in the populations grown in the field, considerable variation in R^2 values was observed. This was probably due to the stage in which the plants were sampled and the limiting environmental conditions in the greenhouse for expression of maximum potential for yield and protein production. The low number of plants and type of leaf sampled (first and second leaves and not the flag leaf) may have been another factor.

V. SUMMARY AND CONCLUSIONS

The main objective of this study was to investigate the relationship between nitrate reductase activity and certain N-yield parameters in four barley cultivars and F_2 segregating plants grown in the field and greenhouse. In Experiment I the treatments consisted of two malting barley cultivars, Larker and Karl; two feed barleys, Steptoe and Benton; and three levels of nitrogen fertilizer which was applied in the form of calcium nitrate at the rates of 0, 60 and 100 + 60 kg N/ha.

Cultivars responded to nitrogen treatments by increasing vegetative growth, yield, percent of protein in grain, and total grain protein per area. Nitrate reductase activity increased with nitrogen fertilizer levels. Differences in levels of NRA among cultivars were also observed. There was a seasonal variation in NRA. It was observed that cultivars may also exhibit differences in "enzyme efficiency" and "transport efficiency". Nitrate reductase activity was causally related to grain yield and protein because its level of activity is an index of potential to reduce nitrogen. Daily input of reduced nitrogen based on NRA assay showed a better association with grain yield and grain protein production than NRA. NRA and daily input of reduced nitrogen from enzyme assay in general showed the highest association with yield and protein production, when measured at stem elongation.

Glutamine synthetase activity apparently was not directly related to yield and grain protein production. Its activity varied among cultivars and within a cultivar with levels of N-fertilizer.

The R^2 between NRA and yield and protein production of F_2 individual plants was variable.

The following conclusions were drawn from this study:

1. Nitrate reductase is related to nitrate-nitrogen and percentage of reduced-nitrogen in plant tissue.
2. Nitrate reductase activity is related to grain yield and grain protein production.
3. The degree of association between NRA and grain yield and grain protein production varies with cultivar and stage of plant development.
4. The highest degrees of association (R^2 values) are detected at the stages of stem elongation and flowering.
5. There are differences in "enzyme efficiency" and "transport efficiency" among cultivars. "Enzyme efficiency" is more important than "transport efficiency" in regard to grain and total grain protein production.
6. Daily input of reduced nitrogen from NRA assay shows better association with yield of grain and grain protein production than NRA.
7. Glutamine synthetase activity at grain filling apparently is not directly related to grain yield and grain protein.
8. For individual F_2 segregating plants, the degree of association between NRA, measured during grain filling, and yield of grain and grain protein production is too variable to be meaningful as a realistic selection tool for either increased yield or grain protein production.
9. By knowing physiological traits such as NRA, "enzyme efficiency" and "transport efficiency", breeders would be better able to make decisions regarding parental combinations for specific objectives in barley breeding.

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APPENDICES

APPENDIX 1

Table 1. Degrees of freedom (DF), Means Squares and F values from ANOVA table for yield of grain, percent protein in grain and total grain protein production per hectare.

		Yield of Grain		Percent of Protein		Total Grain Protein/Area	
SU	DR	MS	F	MS	F	MS	F
Blocks	3	1,411.09		1.39		22,691.85	
N. Fert.	2	36,215.57	113.83**	102.11	88.95**	1,131,447.47	131.65**
Error (a)	6	318.15		1.148		8,594.15	
Cult.	3	4,344.59	14.92**	8.50	38.36**	10,098.07	2.259
Cult. x N-Fert.	6	505.26	1.74	.94	4.24**	10,467.34	2.342
Error (b)	27	291.10		.22		4,469.75	

APPENDIX 2

Table 2. Plant fresh weight at four stages of development and three N-fertilizer levels.

	Tillering	Stem Elongation	Flowering	Grain Filling
0 kg N/ha				
Steptoe	3213	11159	13457	18605
Larker	5081	12358	17428	17966
Benton	3624	10318	12953	18770
Karl	3451	9210	12703	17917
60 kg N/ha				
Steptoe	8490	25161	25981	30485
Larker	9084	35449	35657	34176
Benton	7713	23238	30493	32849
Karl	8240	23534	26639	32097
100 kg N/ha				
Steptoe	8001	308122	39384	38752
Larker	12164	38694	41895	36449
Benton	7777	30460	36785	33057
Karl	9370	28025	39456	38784

APPENDIX 3

Table 3. Degrees of freedom (DF), Mean Squares (MS) and F values from ANOVA table of NRA, nitrate nitrogen and percentage of reduced nitrogen, of four barley cultivars under three N-fertilizer levels and five stages of plant development.

		Nitrate Reductase Activity		Nitrate Nitrogen		Percentage of Reduced N	
SU	DF	MS	F	MS	F	MS	F
N-Fert. x Cult. x Stages	191	1.986		3,117.91.		2.35	
N-Fert. x Cult.	47	.577		2,547.50		0.61	
N-Fert.	11	2.024		10,466.58		2.16	
Blocks	3	3.120	6.475*	715,453.10		1.67	7.08**
N-Fert.	2	5.008	10.395*	55,885,942.85	276.184**	8.66	36.63**
Error (a)	6	0.482		202,350.49		0.24	
Cultivars	3	0.756	10.912**	393,891.91	3.863*	0.87	12.20**
N-Fert. x Cult.	6	0.121	1.753	110,894.34	1.088	0.026	
Error (b)	27	0.0692		101.955.23		0.07	
Stages of Dev.	3	89.561	125.040**	63,716,153.58	162.80**	99.54	935.70**
N-Fert. x Stages	6	0.611	0.854	18,979,844.58	48.493**	0.41	3.82**
Cult. x Stages	9	0.479	0.669	228,049.53	0.58	0.163	1.53
N-Fert. x Cult. x Stages	18	0.064	0.090	416,245.41	1.064	0.05	0.50
Error (c)	108	0.716		391,387.03		0.11	

APPENDIX 4

Table 4. Degrees of freedom (DF), Means Squares (MS) and F values from ANOVA table for nitrate nitrogen (ppm) in plant tissue at five stages of plant development.

		Tillering		Stem Elongation		Flowering		Grain Filling		Maturity	
SU	DF	MS	F	MS	F	MS	F	MS	F	MS	F
Blocks	3	5,832,078		452,674		54,434		73,292		12,990	
N-Fertilizer	2	120,666,403	33.06*	6,926,659	20.61**	2,659,298	57.52**	615,695	10.08	945,767	21.78
Error (a)	6	3,650,253		336,157		46,230		61,093		43,429	
Cult.	3	722,959		398,809	9.63**	33,080	.94	151,440	5.42	6,531	.119
Cult. x N-Fert.	6	1,092,323	2.014	484,835	11.70**	38,444	1.09	128,815	4.61**	28,094	.513
Error (b)	27	542.238		41,432		35,216		27,946		54,772	

APPENDIX 5

Table 5. Degrees of freedom (DF), Mean squares (MS) and F values from ANOVA table for percentage of reduced nitrogen in plant tissue at five stages of plant development.

		Tillering		Stem Elongation		Flowering		Grain Filling		Maturity	
SU	DF	MS	F	MS	F	MS	F	MS	F	MS	F
Blocks	3	2.403		0.288		0.258		2.513		0.006	
N-Fertilizer	2	2.411	4.152	4.426	29.37**	1.788	29.97**	1.237	21.71**	0.411	182.14**
Error (a)	6	0.581		0.151		0.060		0.057		0.002	
Cult.	3	0.755	22.15**	0.358	7.71**	0.072	1.66	0.211	5.32**	0.130	17.59**
Cult. x N-Fert.	6	0.099	2.89*	0.097	2.08	0.011	0.263	0.006	.14	0.026	3.54*
Error (b)	27	0.034		0.046		0.043		0.040		0.007	

APPENDIX 6

Table 6. Degrees of freedom (DF), mean squares (MS) and F values from ANOVA table for total maturity (kg/ha) "Transport efficiency" and total reduced N (kg/ha) at maturity.

		Total N at Maturity		"Transport Efficiency"		Total Reduced N/Area	
SU	DF	MS	F	MS	F	MS	F
Blocks	3	2,861.38		83.07		2863.75	
Fert. L.	2	69,456.35	125.34**	21.18	1.34	69.68	125.77
Error (a)	6	554.16		24.29		554.02	
Cult.	3	890.49	5.64**	202.32	8.33**	451.85	0.02
Cult. x N-Fert.	6	129.51	.821	72.93	3.00*	354.21	0.01
Error (b)	27	157.74		24.29		28,224	

APPENDIX 7

Table 7. Degrees of freedom (DF), means squares (MS) and F values from ANOVA table for nitrate reductase activity at four stages of plant development.

		Tillering		Stem Elongation		Flowering		Grain Filling	
SU	DF	MS	F	MS	F	MS	F	MS	F
Blocks	3	3.186		.275		0.235		21.551	
N. Fert.	2	.258	.89	1.421	37.43**	3.378	5.04	1.783	10.88*
Error (a)	6	.291		0.040		0.670		0.164	
Cult.	3	.150	2.88	.124	3.068**	1.469	24.62*	0.448	7.02**
Cult. x N-Fert.	6	.0123	2.36	.0154	0.382	.154	2.59*	0.133	2.076
Error (b)	27	.0522		.040		.0597		0.064	

APPENDIX 8

Table 8. Degrees of freedom (DF), mean squares (MS) and F values from ANOVA table of daily input of reduced nitrogen based on enzyme assay at four stages of plant development.

		Tillering		Stem Elongation		Flowering		Grain Filling	
SU	DF	MS	F	MS	F	MS	F	MS	F
Blocks	3	2,685,823		4.58		19.73		781.79	
N-Fert.	2	5,899,709	4.77	27.07	19.79**	453.48	10.85*	1,014.46	15.42*
Error (a)	6	1,236,288		4.90		41.78		65.78	
Cult.	3	111,344.69	.97	4.57	30.52**	42.56	7.58*	17.46	1.23
Cult. x N-Fert.	6	197,465.79	1.71	28.85	192.45**	11.18	1.99	13.29	.94
Error (b)	27	115,362.90		.15		5.62		14.16	