CHAPTER VI

KINETICS OF NITRATE UPTAKE AND NITRATE REDUCTASE
IN RELATION TO DWARFISM
CHAPTER VI

6. NITRATE UPTAKE AND NITRATE REDUCTASE ACTIVITIES IN RELATION TO DWARFISM

6.1 REVIEW OF LITERATURE

It is now a common place knowledge that nitrogen in the soils is available for absorption either in the form of nitrate ion or Ammonium, but among them nitrate forms, the main source for the roots of higher plants. Nitrates which are absorbed by the roots are rapidly translocated to leaves where they are either stored or reduced to ammonium through the activity of Nitrate reductase (NR) and Nitrite reductase (NiR). Filner (1969) has summarised these changes.

NITRATE PATHWAY (Filner, P., 1969)
The increased use of nitrogen fertilizers has been a significant factor in increasing productivity of crop plants (Hageman, 1979). Because the energy required for fertilizer nitrogen is costly, there is an obvious need for thorough understanding of the effects of external nitrogen supply on the factors affecting nitrate assimilation and plant growth. Further, excessive doses of nitrogen fertilizer are also known to increase the nitrate content of plants, which causes acute poisoning to cattle, sheep and other livestocks (Wright and Davison, 1964; Te Velde, 1967).

The processes of nitrate uptake, reduction of nitrate and translocation of nitrate or reduced nitrogen are well separated processes both in time and space. Their mutual interactions and ultimate integration are still unclear (Jackson, 1978). The studies involving nitrate uptake, carried out by Heimer et al., (1966); Jackson et al., (1973) and Rao and Rains, (1976) have shown that uptake of nitrate is characterized by a slow rate which grades progressively into a more rapid rate maintained for several hours. The time course studies of nitrate uptake are indicative of an inducible transport system (Breteller et al., 1979; Breteller and Hanisch ten cate, 1980) and it has been demonstrated that accelerated rate of nitrate uptake is countered by the inhibitors of RNA and protein synthesis (Jackson et al., 1973, 1974; Thompkins et al., 1978).

Ben-Zioni et al., (1971) proposed a model according to
which nitrates entering the roots are transported to the shoot where they are reduced and potassium malate was produced. Some of potassium malate was transported to the root system where it was oxidised to give potassium bicarbonate which was subsequently exchanged for potassium nitrate, the reduction of nitrate noticed was stoichiometric to the amount of nitrate transported. Neyra and Hageman (1975) working with Zea mays suggested that other organic acids besides malate could also be involved in nitrate uptake.

Van den Honert and Hooymans (1955) ascertained that nitrate absorption by plants exhibits saturation kinetics. Analogously, Chantarotwong et al., (1976); Doddema et al., (1968), Doddema and Telkamp (1979) and Ioannis et al., (1979) reported that nitrate uptake patterns were consistent with the hypothesis that the ion uptake is mediated by a carrier, in accordance with Michaelis-Menten enzyme kinetics. Huffaker and Rains (1978) however, highlighted that such kinetic analysis may be useful in describing plant responses to nitrate under natural conditions.

The significant correlation between the estimated amount of reduced nitrate supply to the plant (by the in vitro or in vivo nitrate reductase assay) and the actual amount accumulated by the plant (Eilrich and Hageman, 1973; Deckard et al., 1973; Dalling et al., 1975; Brunetti and Hageman, 1976) support the concept that nitrate reduction is the rate limiting step in the assimilation of nitrate to
Nitrate reductase (NADH : Nitrate oxidoreductase, EC 1.6.6.1) - the first enzyme in the reduction series, is a cytosol molybdoenzyme (Ritenour et al., 1967) and is substrate inducible (Beevers and Hageman, 1969; Zielke and Filner, 1970; Kaplan et al., 1974 and Manzano et al., 1976). Garner et al., (1974) proposed a model for the functioning of molybdenum in NR activity. According to model there are changes in oxidation state of Mo (V) and Mo (IV) involved in the nitrate reductase (NR) molecule. The NR is shown to be directly bound to the Mo (V) centre of the enzyme. The coordination between the metal centre and nitrate lowers the activation energy for transfer of electrons.

The extreme sensitivity of this enzyme to a number of physiological, environmental and genetical factors has been reviewed by Beevers and Hageman (1969; Hageman (1979) and Srivastava (1980). Metabolic control also affects NR levels (Liu and Hellebust, 1974; Radin, 1975, 1976, 1977), particularly protein synthesis (Shen, 1969; Jackson et al., 1973, 1974) and carbohydrate supply (Aslam et al., 1973; Goldsmith et al., 1973; Meeker et al., 1974; Aslam and Oaks, 1975; Butz and Jackson, 1977; Zink, 1982) and is repressed by the end products (Smith and Thompson, 1971; Stewart, 1972).

NR is also considered to be factor of prime importance that limits production of cereal grain and grain protein
The activity of NR has been correlated with total reduced nitrogen in the vegetation and grain in wheat (Rilrich and Hageman, 1973), grain yield and grain protein in corn (Deckard et al., 1973), total dry weight accumulation in rye grass (Bowerman and Goodman, 1971), flour and bread quality in wheat and productivity in some other agricultural plants (Reilly, 1976, 1976a, 1976b; Tokarev and Shumnyi, 1976). The enzyme NR at seedling stage has also been considered as a criterion of hybrid vigour in Sorghum (Bhatt, et al., 1979). However, Reed and Hageman (1980, 1980a) pointed out that both rate of nitrate uptake and level of NR activity affect the accumulation of reduced nitrogen in the plant and that, changes in metabolic activities with plant development may significantly affect both these processes thus, highlighting the problem encountered in attempting to develop a simple physiological and biochemical screening criterion useful in identifying superior cultivars at seedling stage.

Genetic regulation of NR is established from the studies on variation in NR levels among the genotypes within species in several plants viz. corn (Zieseri and Hageman, 1962), sorghum (Eck and Hageman, 1974) and from the observations dealing with the high heritability of NR in hybrids and their progenies (Schrader et al., 1966; Warner et al., 1969). These gene enzyme relationship studies of NR disclosed the fact that six genes are involved in the regulation of its synthesis (Cove and Pateman, 1969). Further
correlative studies of NR and nitrate transport (Aslam et al., 1976) gave an indication of co-ordinated regulation and implies that these two activities arise from common or neighbouring genes. Based on the reports - that production of protein can effectively be increased through timely application of appropriate and adequate nitrogen fertilizer and NR can be used as a productivity index of grain and grain protein together with highly heritable nature of the enzyme, Hageman and his associates (1974) aptly suggested that NR can be used as a biochemical criterion for selection of protein rich cultivars for genetic manipulations.

Dwarf plants with their short-stiff straw are highly responsive to fertilizer and it has been shown that NR activity is inversely correlated with plant height at maturity (Vaishnav et al., 1978). Nitrate uptake and nitrate reductase are the vital steps in the nitrate reduction. Thus, it was thought appropriate to undertake studies on kinetics of nitrate uptake and nitrate reductase activity in both wheat and maize cultivars in relation to the physiology of dwarfism and herein the results are presented.

6.2 EXPERIMENTAL

6.2.1 Nitrate uptake

Uniformly germinated seeds of three cultivars each, of wheat and maize, grown in dark, were transferred to 9 cm petri dishes containing 5 ml deionised distilled water. The petri dishes were then transferred to light in a BOD
incubator as described in Chapter II. The seedlings were allowed to grow in distilled water (deionised) for 48 h. Uniformly grown seedlings were selected from each cultivar and used for determining the nitrate uptake kinetics.

From the 1 mM stock solution of potassium nitrate, the following concentrations were made:

1. 0.025 mM  
2. 0.05 mM  
3. 0.1 mM  
4. 0.2 mM  
5. 0.3 mM  
6. 0.4 mM  
7. 0.5 mM  
8. 0.6 mM  
9. 0.7 mM  
10. 0.8 mM  
11. 0.9 mM  
12. 1.0 mM

Twelve ml of these solutions were added to 9 cm petri dishes containing whatman filter paper (No.1). After ten min., 4 ml from the 12 ml solution was pipetted out to serve as the control for nitrate uptake. Ten uniformly grown seedlings of each cultivar were transferred to each set of different concentrations of solutions and kept in light. After 8 h, the left over nitrate was estimated by converting NO to NO\textsubscript{2} following the method of Fishman et al., (1964).

The conversion mixture contained:

\begin{align*}
4.0 \text{ ml test solution} \\
0.2 \text{ ml NaOH (0.1 M)} \\
0.2 \text{ ml CuSO} \text{(0.12\%)} \\
0.2 \text{ ml Hydrazine sulphate (0.2082\%)} \\
4.6 \text{ ml}
\end{align*}

Blanks were also taken in which 0.6 ml DW was added in
place of reducing agent. The mixture was then incubated at 60°C for 30 min. After cooling, 1 ml of the solution from the mixture was added to 1 ml sulphanilamide and 1 ml N-(1-Napthyl) ethylene diamine dihydrochloride (Evans and Nason, 1953) and the pink colour developed was recorded at 540 nm. Standard regression curve was prepared (Fig. 6.1) by using NaNO₂ in different concentrations (viz., 10-50μM). The standard values of concentration versus optical density were computed for linear regression analysis and the following regression equation was obtained.

\[ Y = 0.09252 + 0.01819 X \quad r = 0.9224 \]

The \( K \) values and \( V \) were calculated and expressed as \( m^{-1} \) and \( m^{-1} \) mM and umole. 8h seedling respectively.

The data on the kinetics of nitrate uptake were analysed according to the methods of Michaelis-Menten (1913), Lineweaver and Burk (1934) and Eisenthal and Cornish-Bowden (1974).

The Michaelis-Menten plot gives a rectangular hyperbolic curve from which \( K_m \), the Michaelis constant expressed in units of concentration and \( V_{max} \), the maximum velocity at saturation concentration of the substrate, can be directly determined. The mathematical equation which defines the quantitative relationship between the rate of an enzyme reaction and substrate concentration can be given by the equation:
\[ V_{\text{max}} \left[ \frac{[S]}{K + [S]} \right] \]

However, according to Wilkinson (1961), the Michaelis-Menten plot is statistically biased and cannot be used for complex enzyme reaction. Moreover, the main disadvantage of this method is that, it is not only difficult affair to fit a regression curve through these points but also a time consuming process. And if the regression curve is denied then all the points do not get/receive adequate weightage.

Kinetic data were further analysed by another graphical method of Lineweaver and Burk (1934) involving straight line extrapolations and constant slopes. This method can be used for determining dissociation constants of enzyme-substrate and enzyme-inhibitor compounds and other related constants, when the data are found to be consistent with an assigned mechanisms. A reciprocal of the substrate concentration (s) yields a simple straight line whose slope and ordinate intercept, and which can be used to infer \( K_s \) (Michaelis-dissociation constant) and \( V_{\text{max}} \) (the theoretical velocity). Mathematically, \( K_m \) can be calculated by using the straight line equation:

\[
\frac{1}{V_{\text{max}}} = \frac{1}{K_m [S]} + \frac{1}{V_{\text{max}}} \]

The Lineweaver-Burk plot is particularly useful in interpreting data involving competitive and non-competitive
inhibition and is also applicable to general chemical catalysis. However, the disadvantage of method is the clumping of the points (observed for high substrate concentration) near the Y-axis and thus the variance observed is very large.

Most enzymologists however analyse the results in which Michaelis-Menten equation is obeyed by the method of Eisenthal and Cornish-Bowden (1974), wherein the readings are plotted as lines in parameter space, instead of points. The point of intercept when read on X-axis gives the $K_m$ depending on the number of interactions, the mean and variance from the mean can also be calculated. This method has an added advantage over the other by the fact that it is (i) simple, (ii) require no calculation or mathematical tables, (iii) the kinetic constants can be read directly from the plot.

Results of all the experiments on the kinetics of nitrate uptake, were represented and interpreted by the above given methods (Fig. 6.1, wheat and Fig. 6.3, maize).

6.2.2 Nitrate Reductase

The plant materials were washed with distilled water and dissected into; root, shoot and endosperm in case of seedlings at different stages of germination (Laboratory Experiments) and internodes of fully grown field plants (Field Experiments) as described in Chapter II.

In vivo nitrate reductase activity was estimated by
method of Srivastava (1974). A known amount (500 mg) of plant material was cut into small pieces and incubated in a medium containing 5 ml n-propanol (5% v/v), 2.5 ml phosphate buffer (0.32M, pH 7.4) and 2.5 ml KNO (0.2M). The samples were then vacuum infiltrated and incubated in dark at room temperature. After 45 min., the samples were homogenized in the incubation medium. To the 1 ml of the homogenate, 1 ml of 1% sulphanilamide in 3 N HCl and 1 ml of 0.02% N-(1-Naphthyl) ethylene diamine dihydrochloride solutions were added. After 20 min., the mixture was centrifuged at 10,000 g for 10 min and the absorbance of pink coloured supernatant was recorded at 540 nm. The amount of NO formed was calculated using linear regression equation of standard curve (Fig. 6.1). The enzyme activity is expressed as umole NO produced 45 min \(^{-1}\) (g fresh weight) \(^{-1}\). The experiment was repeated twice with practically similar results.

6.3 OBSERVATIONS

6.3.1 Laboratory Experiments

6.3.1(1) Nitrate Uptake

Graphical representations of the data on nitrate uptake as a function of concentration of the ambient solution in different cultivars of wheat and maize are given in Figs. 6.1 and 6.3 respectively.

In all the cultivars, nitrate uptake showed a hyperbolic saturation kinetics which is similar to the Michaelis-Menten kinetics of enzyme activity. A regression
Fig. 6.1a. Presenting nitrate uptake as a function of a different concentration of nitrate in 48h old seedlings of three cultivars of wheat. A regression line was made from the experimental points and $K_m$ and $V_{max}$ were determined following Michaelis-Menten enzyme kinetics.

1. WL-1562
   \[ Y = 3.7072 + 18.5234X - 14.2986X^2 \]

2. PNC-1
   \[ Y = 2.8490 + 15.9049X - 11.7482X^2 \]

3. C-306
   \[ Y = 3.3487 + 17.5615X - 14.0380X^2 \]

Fig. 6.1b. Showing graphical representation of the data analysed by method of Line Weaver and Burk Plot - involving straight line extrapolations and constant slopes. A reciprocal of the substrate concentration $[S]$ yields a simple straight line whose slope and ordinate intercept and which can be used to infer $K_s$ (Michaelis-dissociation constant) and $V_{max}$ - the theoretical velocity.

Fig. 6.1c-e. Displaying the graphical representation of the data analysed by method of Eisenthal and Cornish-Bowden plot, wherein reading are plotted as lines in parameter space, instead of points. And the points of intercept when read on the X-axis gives the $K_m$.

Fig. 6.1f. Standard regression curve of $\text{NaNO}_2$.

Table 61. Kinetic values of nitrate uptake.

<table>
<thead>
<tr>
<th>Cultivar Number</th>
<th>Cultivar Name</th>
<th>$V_{max}$ $\mu\text{M/hr/seedling}$</th>
<th>$V_{max}$ $\mu\text{M/hr/seedling}$</th>
<th>$K_m$ $\text{mM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WL-1562</td>
<td>9.673740</td>
<td>1.209218</td>
<td>0.064163</td>
</tr>
<tr>
<td>2</td>
<td>PNC-1</td>
<td>8.225804</td>
<td>1.028226</td>
<td>0.084787</td>
</tr>
<tr>
<td>3</td>
<td>C-306</td>
<td>8.832648</td>
<td>1.04081</td>
<td>0.064074</td>
</tr>
</tbody>
</table>
Wheat

Fig. 6.1

Standard Curve
Sodium Nitrite (NaNO₂)

\[ Y = 0.0925 + 0.01819X \]

\[ r = 0.9224 \]
Fig. 6.2a Regression analysis showing $V_{\text{max}}$ as a function of plant height at maturity and the correlation is significant at 5% $P$ level.

Fig. 6.2b. Showing the relationship between integrated soluble protein values of all the internodes versus mature plant height in three cultivars of wheat grown in field.

Fig. 6.2c. Exhibiting the relationship between integrated values of soluble protein with integrated values of \textit{in vivo} nitrate reductase of all internodes of each of the three wheat cultivars grown in the field. The correlation is significant at 5% $P$ level.
Wheat

**Vmax Vs Plant Height**

Vmax (μM NaNO₂/8 Hours/Seedling)

\[ Y = 30.77443 - 0.42062 X + 0.00196 X^2 \]

\[ r = -0.62927 \]

Plant Height at Maturity (cm)

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

Wheat

**Soluble Proteins Vs. Plant Height**

mg Protein / g Fresh Weight

\[ Y = 46.77353 - 0.60578 X + 0.00250 X^2 \]

\[ r = -0.94735 \]

Plant Height at Maturity (cm)

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

Wheat

**Soluble Proteins Vs. Nitrater Reductase**

μMole NO₂ Produced/45 Min/g Fresh Weight

\[ y = -5607.917 + 957.975 - 38.12921X \]

\[ r = 0.63426 \]

mg Protein / g Fresh Weight

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated
Fig. 6.3a Presenting nitrate uptake as a function of a different concentration of nitrate in 48h old seedlings of three cultivars of maize. A regression line was made from the experimental points and $K_m$ and $V_{max}$ were determined following Michaelis-Menten enzyme kinetics.

1. J-202
   \[ Y = 5.9761130.2758X - 29.6153X^2 \]

2. Vijay
   \[ Y = 3.3492466 4147X - 51.5110X^2 \]

3. African
   \[ Y = 2 8927+37.9556X - 16.1119X^2 \]

Fig. 6.3b. Showing graphical representation of the data analysed by method of Line Weaver and Burk Plot — involving straight line extrapolations and constant slopes. A reciprocal of the substrate concentration [S] yields a simple straight line whose slope and ordinate intercept and which can be used to infer $K_m$ (Michaelis-dissociation constant) and $V_{max}$ — the theoretical velocity.

Fig. 6.3c-e. Displaying the graphical representation of the data analysed by method of Eisenthal and Cornish-Bowden plot, wherein reading are plotted as lines in parameter space, instead of points. And the points of intercept when read on the X-axis gives the $K_m$.

Fig. 6.3f. Standard regression curve of protien.

### Table 62. Kinetic values of nitrate uptake.

<table>
<thead>
<tr>
<th>Cultivar Number</th>
<th>Cultivar Number</th>
<th>$V_{max}$ μM/8hrs/seedling</th>
<th>$V_{max}$ μM/hr/seedling</th>
<th>$K_m$ mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>J-202</td>
<td>13.710175</td>
<td>1.713772</td>
<td>0.029908</td>
</tr>
<tr>
<td>2.</td>
<td>VIJAY</td>
<td>24.654060</td>
<td>3.081758</td>
<td>0.153489</td>
</tr>
<tr>
<td>3.</td>
<td>AFRICAN</td>
<td>21.665564</td>
<td>2.708196</td>
<td>0.235712</td>
</tr>
</tbody>
</table>
line was calculated for each cultivar from experimental points. The $K_m$ and $V_{\text{max}}$ values were determined from calculated line and presented in Tables 6.1 (wheat) and 6.2 (maize).

On the basis of $K_m$ value these cultivars were divided into three categories:

(i) Cultivars with low $K_m$ values (cal. 0.064 mM). In this category two cultivars of wheat viz., WL-1562 and C-306, and one cultivar of maize viz., J-202 are included.

(ii) Cultivars with medium $K_m$ values (cal. 0.154 mM). This category includes cultivars PNC-1 (wheat) and Vijay (maize).

(iii) Cultivars with high $K_m$ values (cal. 0.234 mM). This category includes cultivar African (maize).

On the basis of $V_{\text{max}}$ these cultivars of wheat and maize were divided into two categories:

(i) Cultivars with low $V_{\text{max}}$ (cal. 8.0 - 14.0 umole/8h/seedling). This group includes cultivars: WL-1562, PNC-1, C-306 (wheat) and J-202 (maize).

(ii) Cultivars with high $V_{\text{max}}$ (cal. 21.0 - 25.0 umole/8h/seedling). This category includes cultivars: Vijay and African of maize.

$V_{\text{max}}$ values when plotted against plant height at maturity (Fig. 6.2) showed a significant (at 5% P) inverse relationship in wheat, suggesting thereby that the dwarf
cultivars can absorb nitrate at a much faster rate than the tall plants. However this relationship was direct (significant at 5 % P) in maize (Fig.6.4).

The soluble proteins (integrated values of all the internodes) versus plant height at maturity in wheat (Fig. 6.2) showed an inverse relationship in wheat (significant at 1 % P), whereas as in maize (Fig. 6.4) a direct significant relationship was observed. However, the integrated NR value of all the internodes versus soluble proteins (integrated values) showed a significant (at 5 % P) direct correlation in wheat (Fig. 6.2) while in maize very low inverse correlation was noticed.

6.3.1(2) Nitrate Reductase Activity

Data on in vivo NR activity in root, shoot and endosperm of wheat and maize seedlings are presented in Figs. 6.5 and 6.7 and their statistical treatments are given in Figs. 6.6 and 6.8, respectively.

From the data, it is clear that NR activity in root and shoot of wheat and maize decreased with the advancement in seedling age; while in endosperm, an upsurge in its activity in general, was recorded. The regression analysis at different stages of growth indicated an inverse relationship between NR activity and plant height at maturity, in root and shoot of both wheat and maize, while in endosperm the activity was always positively correlated with plant height at maturity.
Fig. 6.4a. Regression analysis showing $V_{\text{max}}$ as a function of plant height at maturity and the correlation is significant at 5 % P level.

Fig. 6.4b. Showing the relationship between integrated soluble protein values of all the internodes versus mature plant height in three cultivars of maize grown in field.

Fig. 6.4c. Exhibiting the relationship between integrated values of soluble protein with integrated values of in vivo nitrate reductase of all internodes of each of the three maize cultivars grown in the field.
Maize

**Vmax Vs. Plant Height**

Vmax (μM NaNO₃/0 Hours/Seedling)

\[
Y = -73.59690 + 0.95244X - 0.00230X^2
\]

\[r = 0.73295\]

**Plant Height at Maturity (cm)**

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated

**Maize**

Soluble Proteins Vs. Plant Height

mg Protein / g Fresh Weight

\[
Y = 165.13358 - 1.25806X + 0.00413X^2
\]

\[r = 0.9263\]

**Maize**

Soluble Proteins Vs. Nitrate Reductase

μMole NO₂ Produced/45 Min/g Fresh Weight

\[
Y = -34149.252 + 840.712X - 4.773X^2
\]

\[r = -0.17539\]

--- Y-Estimated

--- Y-Estimated

--- Y-Estimated
Fig. 6.5 *In vivo* nitrate reductase activity in root, shoot and endosperm at early stages of seedling growth of three wheat cultivars varying in their plant height at maturity. The activity is expressed as $\mu$ mole NO$_2$ produced hr$^{-1}$ (g fresh weight)$^{-1}$. 
Wheat
Nitrate Reductase
Root.

Shoot

Endosperm

Fig. 8.5
Fig. 6.6. Showing the relationship between \textit{nitrate reductase} (IN VIVO) activity in root, shoot and endosperm (Y); plant height at maturity of three \textit{wheat} cultivars and four growth periods \((X_2)\). Regression lines 1, 2, 3 and 4 represent the periods after germination i.e., 24, 48, 72 and 96 hours respectively. Regression equations and values of coefficient of correlation alongwith their significance are given below.

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>Coefficient of Correlation ((r))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Root</strong></td>
<td></td>
</tr>
<tr>
<td>(Y = 7725.70450 - 4.70886 X_1 - 163.20769 X_2 + 0.00012 X_1^2 + 0.96646 X_2^2)</td>
<td>(r_{X_1Y} = -0.06151) (r_{X_2Y} = -0.90416)** (r_{X_2Y}^2 = -0.06139) (r_{X_2Y}^2 = -0.82010)**</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.97901)</td>
</tr>
<tr>
<td><strong>2. Shoot</strong></td>
<td></td>
</tr>
<tr>
<td>(Y = 1046.33542 + 32.57844 X_1 - 67.20966 X_2 - 0.16904 X_1^2 + 0.44305 X_2^2)</td>
<td>(r_{X_1Y} = -0.07437) (r_{X_2Y} = -0.78636)** (r_{X_2Y}^2 = -0.07940) (r_{X_2Y}^2 = -0.68030)*</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.91434)</td>
</tr>
<tr>
<td><strong>3. Endosperm</strong></td>
<td></td>
</tr>
<tr>
<td>(Y = -1522.72058 + 13.63903 X_1 + 35.16482 X_2 - 0.02878 X_1^2 - 0.21713 X_2^2)</td>
<td>(r_{X_1Y} = 0.40611) (r_{X_2Y} = 0.69456)* (r_{X_2Y}^2 = 0.40412) (r_{X_2Y}^2 = 0.62113)*</td>
</tr>
<tr>
<td></td>
<td>(R^2 = 0.77401)</td>
</tr>
</tbody>
</table>

* Significance at 5%
** Significance at 1%
Wheat
Nitrate Reductase
umoles NO₃ Produced/45 Min/g Fr.Wt.

Plant Height at Maturity(cm)

Root

(Thousands)

Shoot

Endosperm

Wheat
Nitrate Reductase
umoles NO₃ Produced/45 Min/g Fr.Wt.

Plant Height at Maturity(cm)

(1) 24-Hours  (2) 48-Hours  (3) 72-Hours  (4) 96-Hours

Fig. 6.6
Fig. 6.7. *In vivo* nitrate reductase activity in root, shoot and endosperm at early stages of seedling growth of three maize cultivars varying in their plant height at maturity. The activity is expressed as $\mu$ mole NO$_2$ produced hr$^{-1}$ (g fresh weight)$^{-1}$. 
Maize
Nitrate Reductase
Root

Shoot

Endosperm

Fig. 6.7
Fig. 6.8. Presenting the relationship between nitrate reductase (IN VIVO) activity in root, shoot and endosperm (Y); plant height at maturity of three *maize* cultivars and four growth periods (X).

Regression lines 1, 2, 3 and 4 denote the periods after germination i.e., 24, 48, 72 and 96 hours respectively. Regression equations and values of coefficient of correlation along with their significance are given below.

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>Coefficient of Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Root</strong></td>
<td></td>
</tr>
<tr>
<td>$Y = 753.19266 - 0.04412X_1 - 1.80109X_2 - 0.00228X_1^2 - 0.02565X_2^2$</td>
<td>$r_{X_1 Y} = -0.24306$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_2 Y} = -0.79019^{*\star}$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_1^2 Y} = -0.24379$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_2^2 Y} = -0.79354^{*\star}$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.69178$</td>
</tr>
<tr>
<td><strong>2. Shoot</strong></td>
<td></td>
</tr>
<tr>
<td>$Y = 541.62728 - 2.08996X_1 + 1.59318X_2 + 0.00302X_1^2 - 0.02312X_2^2$</td>
<td>$r_{X_1 Y} = -0.74946^{*\star}$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_2 Y} = -0.57217^*$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_1^2 Y} = -0.74096^{*\star}$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_2^2 Y} = -0.60555^*$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.95238$</td>
</tr>
<tr>
<td><strong>3. Endosperm</strong></td>
<td></td>
</tr>
<tr>
<td>$Y = -345.11802 + 4.15761X_1 - 1.47106X_2 - 0.00778X_1^2 + 0.06621X_2^2$</td>
<td>$r_{X_1 Y} = 0.27166$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_2 Y} = 0.89449^{*\star}$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_1^2 Y} = 0.26633$</td>
</tr>
<tr>
<td></td>
<td>$r_{X_2^2 Y} = 0.91509^{*\star}$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.91546$</td>
</tr>
</tbody>
</table>

* *Significance at 5%*

** **Significance at 1%**
6.3.2 Field Experiments

6.3.2(1) Nitrate Reductase Activity

Changes in the in vivo nitrate reductase activity of different internodes of field grown plants of wheat and maize are presented in Figs. 6.9a and 6.10a, respectively.

It is clear from the figure that lower internodes had low activity of NR and the activity increased towards the apex. The data plotted against internode number revealed such relationship (Figs. 6.9b and 6.10b).

NR activity when plotted against internode length (Figs. 6.9c and 6.10c) recorded a direct relationship. Young internodes however, possessed maximum activity.

6.4 DISCUSSION

In the present laboratory experiments, nitrate uptake in all the cultivars of wheat and maize showed saturation kinetics (Figs. 6.1 and 6.3). Similar saturation kinetics for nitrate uptake has been described by a number of workers (Jackson et al., 1973, 1974; Rao and Rains, 1976; Chantarotwong et al., 1976; Clement et al., 1978; Ioannis et al., 1979; Krishanan, 1982).

The kinetics constants, \( K \) and \( V \) (Tables 6.1 & 6.2) recorded low \( K \) values (0.064 mM) in cultivars; WL-1562 and C-306 of wheat and J-202 of maize, suggest that these cultivars are more efficient in absorbing nitrate from low nitrogen concentrations. Cultivar African (of maize) with
Fig. 6.9a. Distribution of nitrate reductase (IN VIVO) activity in different internodes of three wheat cultivars grown in the field. The activity is expressed as \( \mu \text{mole NO}_2\text{ produced/45min/g fresh wi} \). Fig. 6.9b and fig. 6.9c represent the relationship (curvilinear) between activity (Y) as a function of internode number and length (X) respectively. The regression equations and values of coefficient of correlation alongwith their significance given below.

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>Coefficient of Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>b.1. WL-1562</strong></td>
<td></td>
</tr>
<tr>
<td>( X = 135.6488 - 70.4014 X + 15.0363 X^2 )</td>
<td>( r_{xy} = 0.3330 )</td>
</tr>
<tr>
<td>( R^2 = 0.9887 )</td>
<td>( r_{x^2y} = 0.4928 )</td>
</tr>
<tr>
<td><strong>2. PNC-1</strong></td>
<td></td>
</tr>
<tr>
<td>( Y = 70.2243 - 7.5045 X + 2.6818 X^2 )</td>
<td>( r_{xy} = 0.9162^* )</td>
</tr>
<tr>
<td>( R^2 = 0.9780 )</td>
<td>( r_{x^2y} = 0.9674^* )</td>
</tr>
<tr>
<td><strong>3. C-306</strong></td>
<td></td>
</tr>
<tr>
<td>( Y = 38.8127 - 7.4263 X + 3.6479 X^2 )</td>
<td>( r_{xy} = 0.9569^* )</td>
</tr>
<tr>
<td>( R^2 = 0.9990 )</td>
<td>( r_{x^2y} = 0.9928^{**} )</td>
</tr>
<tr>
<td><strong>c.1. WL-1562</strong></td>
<td></td>
</tr>
<tr>
<td>( Y = 121.3282 - 12.0539 X + 0.5513 X )</td>
<td>( r_{xy} = 0.4817 )</td>
</tr>
<tr>
<td>( R^2 = 0.8992 )</td>
<td>( r_{x^2y} = 0.6158 )</td>
</tr>
<tr>
<td><strong>2. PNC-1</strong></td>
<td></td>
</tr>
<tr>
<td>( Y = 60.0311 + 0.7137 X + 0.0087 X^2 )</td>
<td>( r_{xy} = 0.9769^* )</td>
</tr>
<tr>
<td>( R^2 = 0.9562 )</td>
<td>( r_{x^2y} = 0.9713^* )</td>
</tr>
<tr>
<td><strong>3. C-306</strong></td>
<td></td>
</tr>
<tr>
<td>( Y = 34.0837 - 0.3579 X + 0.0524 X^2 )</td>
<td>( r_{xy} = 0.9703^* )</td>
</tr>
<tr>
<td>( R^2 = 0.9862 )</td>
<td>( r_{x^2y} = 0.9924^* )</td>
</tr>
</tbody>
</table>

* Significance at 5%
** Significance at 1%
Wheat Nitrate Reductase

(b) Wheat Nitrate Reductase

(b) Wheat Nitrate Reductase

(c) Wheat Nitrate Reductase

Fig. 6.9
Fig. 6. Comparison of nitrate reductase (IN VIVO) activity in different internodes of three maize cultivars grown in the field. The activity is expressed as \( \mu \text{mole NO}_2 \text{ produced/45 min/g fresh wt} \). Fig. 10b and fig. 10c represent the relationship (curvilinear) between activity (Y) as a function of internode number and length (X) respectively. The regression equations and values of coefficient of correlation along with their significance given below.

| Regression Equation | Coefficient of Correlation (r)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>b.1. J-202</td>
<td></td>
</tr>
<tr>
<td>( X = 89.6177 - 3.8113 X + 0.8966 X^2 )</td>
<td>( r_{XY} = 0.4572 )</td>
</tr>
<tr>
<td>( R^2 = 0.2345 )</td>
<td>( r_{X^2Y} = 0.4816 )</td>
</tr>
</tbody>
</table>

2. VIJAY

| Regression Equation | Coefficient of Correlation (r)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y = 295.5007 - 41.1756 X + 2.6540 X^2 )</td>
<td>( r_{XY} = -0.2403 )</td>
</tr>
<tr>
<td>( R^2 = 0.3345 )</td>
<td>( r_{X^2Y} = -0.1125 )</td>
</tr>
</tbody>
</table>

3. AFRICAN

| Regression Equation | Coefficient of Correlation (r)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y = 99.1577 - 0.2898 X + 0.0561 X^2 )</td>
<td>( r_{XY} = 0.0702 )</td>
</tr>
<tr>
<td>( R^2 = 0.0055 )</td>
<td>( r_{X^2Y} = 0.0741 )</td>
</tr>
</tbody>
</table>

| Regression Equation | Coefficient of Correlation (r)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1. J-202</td>
<td></td>
</tr>
<tr>
<td>( Y = 117.2709 - 12.6112 X + 1.1875 X^2 )</td>
<td>( r_{XY} = 0.3937 )</td>
</tr>
<tr>
<td>( R^2 = 0.2025 )</td>
<td>( r_{X^2Y} = 0.4281 )</td>
</tr>
</tbody>
</table>

2. VIJAY

| Regression Equation | Coefficient of Correlation (r)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y = 432.2499 - 55.4809 X + 2.6791 X^2 )</td>
<td>( r_{XY} = -0.3783 )</td>
</tr>
<tr>
<td>( R^2 = 0.3083 )</td>
<td>( r_{X^2Y} = -0.3090 )</td>
</tr>
</tbody>
</table>

3. AFRICAN

| Regression Equation | Coefficient of Correlation (r)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y = 94.2361 + 1.3278 X - 0.0535 X^2 )</td>
<td>( r_{XY} = 0.0186 )</td>
</tr>
<tr>
<td>( R^2 = 0.0010 )</td>
<td>( r_{X^2Y} = 0.0134 )</td>
</tr>
</tbody>
</table>

* Significance at 5%
* * Significance at 1%
Maize Nitrate Reductase

![Graph of Nitrate Reductase activity across internode number and plant height at maturity.](image)

**Maize Nitrate Reductase**

- umoles NO₂ Produced/45 Min./g Fr.Wt.
- Internode Number
- Plant Height at Maturity (cm)

**Maize Nitrate Reductase**

- umoles NO₂ Produced/45 Min./g Fr.Wt.
- Internode Number
- Internode Length

*(1) J-602  (2) VIAT  (3) AFRICAN*

*Fig. 6.10*
higher K value is the least efficient cultivar in acquiring nitrate at low nitrate fertility. In the crop fields however, K values may not be of much significance because of excessive fertilizer doses which is a common practice these days. Instead, probably V may be an important parameter for nitrate uptake. Reed and Hageman (1980a) concluded that nitrate uptake, its flux and NR activity, affect the accumulation of reduced nitrogen in maize. In the present study also, such correlation was clearly evident in wheat, wherein the potential for nitrate uptake (V) and reduced nitrogen (as soluble protein) recorded an inverse relationship with plant height. In maize however, the potential for nitrate uptake (V) and reduced nitrogen (soluble protein) showed a direct correlation with plant height at maturity.

From the results presented (Laboratory Experiments) in Figs. 6.5 and 6.7, it is evident that during the initial period of growth, endosperm had higher levels of NR activity, followed by root, and shoot however, recorded the minimum levels in both wheat and maize. Regulation of NR activity has been shown to be under the metabolic control (Frith, 1972, Oaks et al., 1977) particularly protein synthesis (Beevers et al., 1965; Shen, 1969) as well as carbohydrate supply (Goldsmith et al., 1973; Aslam and Oak, 1975). Endosperm is the major source of the reserve food material during initial seedling growth and root is the first organ initiated during germination. Higher NR activity in endosperm and root
thereby, may be mainly due to the active metabolic state of these organs. In a study on maize seedlings, Wallace (1973) reported that before leaf expansion, NR activity was mainly present in the scutellum and root. After leaf expansion, more than 90% of the NR activity was in the shoot, mainly in the blade and a marked decrease occurred in the level of enzyme in scutellum and root. Wallace (1973) therefore, concluded that a bulk of nitrate assimilation occurs at the site of available carbohydrate source, which before expansion was present in the scutellum and after expansion, the leaves photosynthetically synthesized it.

Nitrate reductase activity in the internodes of field grown plants of wheat and maize, showed a basipetal distribution (Figs. 6.9b and 6.10b). Similar basipetal patterns of NR activity have also been reported by Bilal and Rains (1973); Blackwood and Hallam (1979); Krishnan (1982). It has been proposed that high metabolic activity and supply of photosynthates for respiration (Aslam et al., 1973) may be important for the basipetal distribution.

NR activity when plotted against internodal length revealed a direct relationship (Figs. 6.9c and 6.10c). Further, NR activity in root and shoot of laboratory grown seedlings recorded an inverse relationship with plant height at maturity (Figs. 6.5 and 6.8). Earlier work from this laboratory (Vaishnav et al., 1978) has also shown an inverse relationship between plant height and integrated nitrate
reductase activity (in flag leaf) for the whole growing season in *Sorghum*. Furthermore, it was also suggested that NR activity at seedling stages may serve as a criterion for hybrid vigour in *Sorghum* (Bhatt *et al.*, 1979).

The association of higher NR activity with dwarfism and its direct correlation with reduced nitrogen (as soluble protein) reported in present work, is of great practical utility (Fig. 6.2). It is therefore, suggested that both in wheat and maize, nitrate reductase activity probably is the most important rate limiting step in the assimilation of nitrate. High doses of nitrogen fertilizers are generally recommended for maximum production and it has been a significant factor in increasing productivity of crop plants. However, in number of cases heavy fertilization is less effective since it causes excessive vegetative growth, which in turn brings about lodging in plants. Eilrich and Hageman (1973) concluded that, the full potential for increasing yield via nitrate reductase activity through nitrogen fertilizers, depends upon their utilization by cultivars that resist lodging. Further, excessive doses of nitrogen fertilizers are also known to increase the nitrate content of plants, which causes recurrent signs of poisoning in cattle and other livestock (Gilbert *et al.*, 1946; Te Velde, 1967).

Differences in nitrate accumulations have been found between plant species (Brown and Smith, 1966; Liebenow, 1971) and between plant varieties (Gul and Kolp, 1960; Barker, *et al.*, 1971). Dwarf wheat and maize plants characterised to
possess high NR activity and protein levels together with reduced lodging, suggest that the selection of these plants for genetic manipulation may prove promising in producing high yielding protein rich cultivars. Furthermore, low requirement of soil nitrate by these cultivars may itself prove better in nitrogen economy and nitrate pollution.