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# Nitrate Uptake and Assimilation Efficiency of Excised Leaves of Barely (C3) and Maize (C4) Plants. I) Effect of Temperature on Nitrate Uptake and Assimilation

**M.O.El_Mohtasem**

Prof. of plant nutrition & soil fertility Soil dept. Fuclty of agr. Cairo,Egypt

Osam1@yahoo.com

**ABSTRACT**

Nutrient assimilation by seedling is an early indicator of subsequent plant growth and development. This study was conducted to compare the effect of temperature on NO3 uptake and reduction by excised leaves of Barley (C3) and Maize (C4) plants with and without source of energy supply. The seedling Barley ( Hordeum Vulgare L.CV UC 337) and Maize ( Zea Mays L.CV pioneer 3162 ) were sown in vermiculate in pots kepts in growth chamber at 25°C. The tip 10 cm portion of leaves excised and placed in small glass vials containing 8 ml from,3.0,6.0 and10 mM of KNO3 solution with and without sucrose. The temperature treatments were 15,20,25,30 and 35°C. Net NO3 uptake were determined by following it`s depletion from uptake solution that initially contained the same concentration of NO3 as the induction solution. NO3 uptake by both C4 and C3 leaves was increased as NO3 level and temperature increased. While C3 leaves(Barley) was more efficient than C4 leaves ( Maize) in NO3 uptake at all NO3 concentration for NO3 reduction percent (assimilation),C4 (Maize) leaves were efficience than C3 (Barley) plants, and NO3 reduction percent increase with increasing the temperature until 25°C then decreased. The addition of sucrose by 1% to the medium as energy source increased the NO3 reduction percent in both C3 and C4 leaves.

## INTRODUCTION

NO3 uptake & accumulation are influenced by many environmental factors as respiration ( Willis & Yemm ,1955 ) , and strongly affected by temperature (Ezeta & Jackson 1975 ).Nitrate accumulation in plant tissues is response to increasing ambient nitrate concentrations ( Chamtarotwong et al ,1976 ). Nitrate uptake affected by low light (Cantliffe ,1972). Also NO3 uptake & reduction by plants differed from ( C3 ) plants to (C4 ) plants,plants with C4 (Maize) photosynthesis produce more dry matter per mg nitrogen utilized than plants with C3 (Barley) metabolism ( Brown, 1978).Oaks(1994) pointed out that uptake of nitrate as also it`s accumulation was greater in Barley than in Maize. While conversion of of NO3 –N to protein –N was greater in Maize. Lawlor et al (1980) found that leaves grown at the lower temperature, especially with more nitrate , contained much more soluble protein,nitrate reductase & free amino acids per leaf unit area than plants grown in warmer conditions. High temperature affect photosynthesis by altering the excitalion energy distribution by chaning the structure of thylakoids (Berry & Bjorkman 1980),and by chaning the activity of the calvin cycle and other processes such as photorespiration and product synthesis.Also the changes in temperature induce

variations in the activity of enzymes & that because of their different Q10 values (Pollock & Rees,1975).

The aims of this work was to study the effects of ambient nitrate concentration, temperature & the addition of sucrose on the processes of nitrate uptake & reduction by Barley (C3 ) and Maize (C4) plants.

**MATERIALS and METHODS**

Barley ( Hordeum Vulgare L.CV.337) and Maize (Zea maize L.CV. Pioneer 3162) seeds were sown in vermiculite as growth media in pots. The pots kept in growth chamber at 25°C in the darkness for 7 days & then in continous light ( 500 U.E m 25-1) for anther 3 days. During the light period, plants were irrigated with N-free, one fourth strength Hoaglan nutrients solution (Hoagland & Arnon ,1950). The seedlings were irrigated with 10 uM potassium nitrate ,24 h prior to the experiments. The tip 10.0cm portion of leaves were excised & placed in small glass vials containing 8.0ml of NO3 as K NO3 (without and with sucrose) ,kept in growth chamber in light and different temperature treatments 15,20,25,30 and 35°C . After 8.0 h uptake period, leaves were removed from the solution, rinsed in water & frozen until NO3 extracted. In vivo nitrate reduction was determined by subtracting the amount of nitrate present in the leaves from that disappeared from vial. Nitrate from excised leaves was extracted by grinding the tissues in mortar & pestle in liquid nitrogen, adding deionized water and centrifugation at 10.000 RPM for 10 minutes. The nitrate in solution was determined using HPLC. In vivo reduction was determined by subtracting the nitrate concentration of the tissue at time (t) from the initial nitrate concentration of the tissues at time (t0) plus the amount of nitrate taken up (Chantarotwong et al,1976). PH did not change significantly during the experiments. Each treatment contained three replicates, and each experiment was replicated three times. The results of the three experiments were then combined for statistical analysis.

**RESULTS**

**NO3 Uptake**

Response curves of NO3 uptake , reduction & reduction percent to increasing concentrations of ambient nitrate constructed for seedling equilibrated in a solution of 0.1 mM for 24 hr. before uptake studies began [Table (1),Figure(1&2)]. Nitrate uptake was linear with increasing NO3 ambient concentration with all temperature treatments in both Barley & Maize plants . NO3 uptake was higher in Barley than Maize at all NO3 ambient concentration. (OAKS et al,1994) found that NO3 uptake was higher in Barley than Maize. For sucrose addition to the nutrient solution [Table (2), figure (3&4)] , the data shows that this addition increased NO3

uptake only with high ambient concentration,especially in corn plants. [Table (1), figure (1&2)] show's that increasing the temperature increased the NO3 uptake in both plants. This relation is more clear in Barley than Maize but increasing the temperature over 30 C the NO3 uptake in Barley decreased . Since nitrate uptake are largely dependent upon respiration, their response to increasing temperature as expected.

**NO**3 **Reduction**

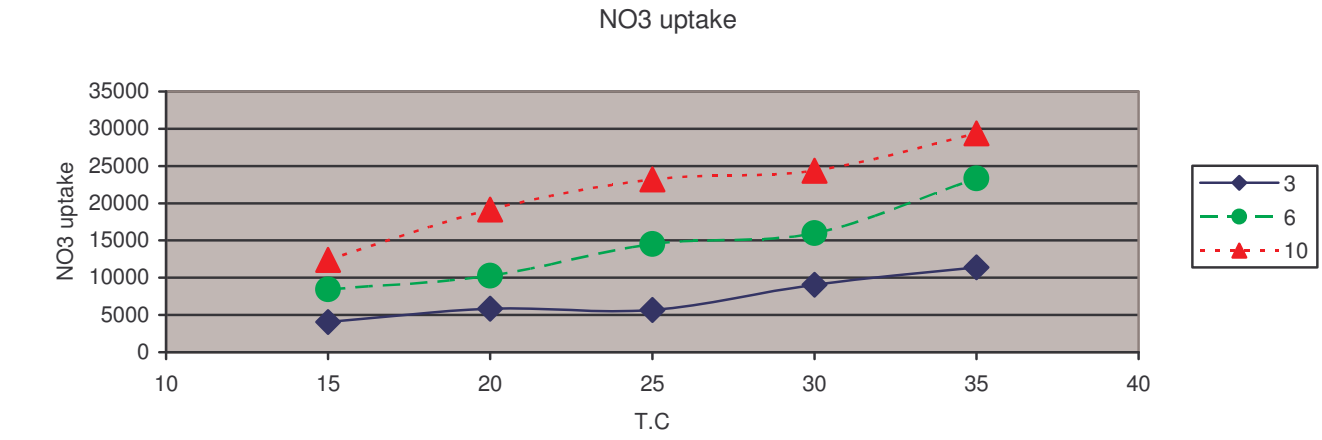
In Vivo,reduction was determined by subtracting the nitrate concentration of the tissues at time ( t ) from the initial nitrate concentration of tissues at time ( t0 ) plus the amount of nitrate taken up( Chantarotwong et al,1976 ). The data presented in [Table (1), figure (1&2)] shows that NO3 reduction increased with increasing ambient NO3 concentration .Also the values of NO3 reduction were higher in Maize than Barley leaves. For the effect of temperature , in Maize leaves NO3 reduction increased with increasing the temperature at all NO3 levels , while in Barley NO3 reduction increased with increasing the temperature until 25 C,while increasing the temperature over 25 C the NO3 reduction increased . For sucrose treatment [Table (2),figure (3&4)] , the data shows that in Maize the effect of sucrose on increasing NO3 reduction was observed in low concecntration (3 mM). while in Barley it's increased NO3 reduction in high concentration (6,10 mM)

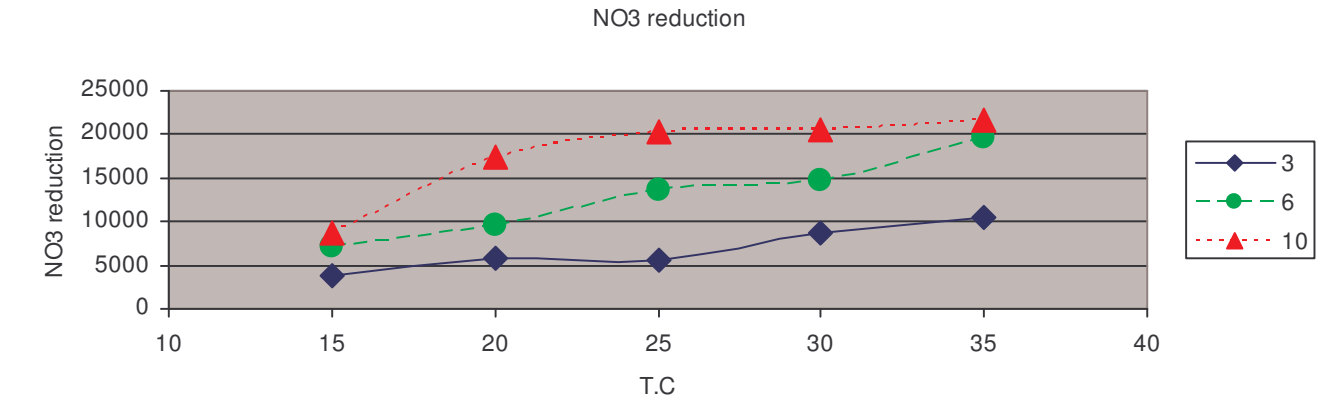
**NO**3 **Reduction Percent (%) :**

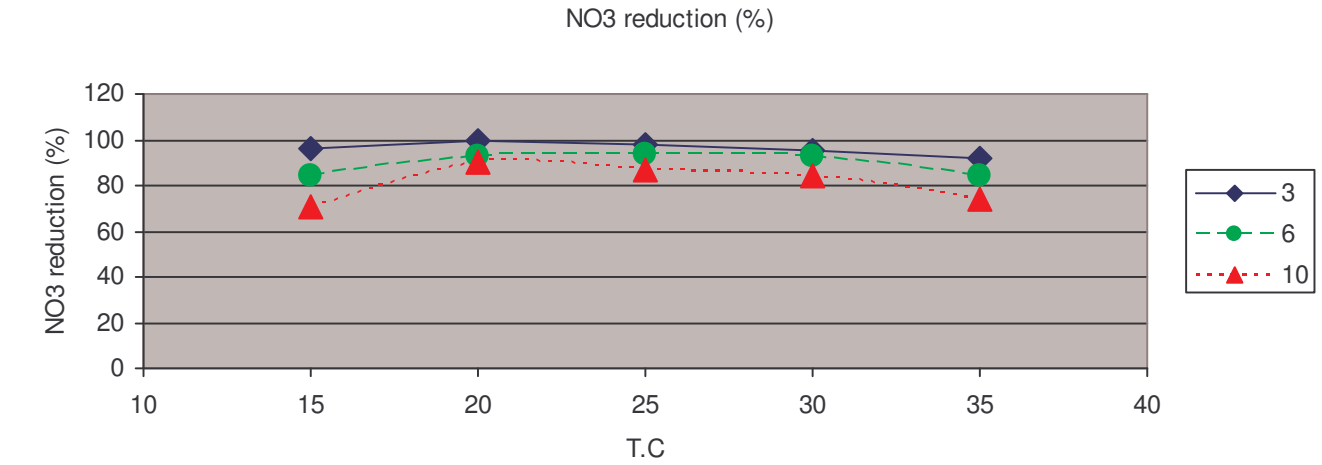
In both Maize and Barley leaves, increasing ambient NO3 concentration decreased the percentage of NO3 reduction ( with or without sucrose ). For effect of temperature on NO3 reduction percent,increasing temperature until 25°C increased reduction percent,while increasing temperature over 25°C caused a decrease in NO3 reduction percent in both Barley and Maize plants [Table (1),figure (1&2)].This effect of high temperature is more pounced in Barley than Maize leaves. This effect due to the effect of high temperature on nitrate reductase enzyme. Also Brown,1978 found that plants with C4 photosynthesis produce more dry matter per mg nitrogen utilized than plants with C3 metabolism. It has been recently reported in spite of a saturating N supply, the youngest mature leaves of Corn contained little of NO3 reserve (Foyer et al 1998 )

Table (1) effect of NO3 concentration & temperature on NO3 uptake,reduction & reduction (%)

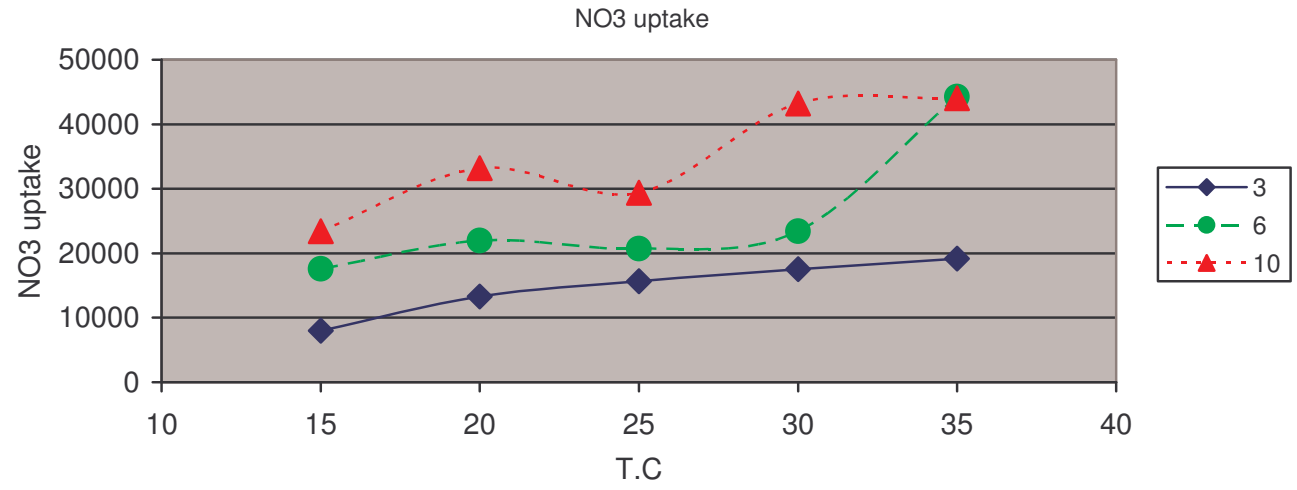
| NO3 Conc.(m.mole) | T.C° | Corn | | | Barley | | |
|---|---|---|---|---|---|---|---|
| | | NO3 uptake uM | NO3 red. | NO3 red. (%) | NO3 uptake uM | NO3 red. | NO3 red. (%) |
| 3.0 | 15.0 | 4028.5 | 3861 | 95.84 | 8010.9 | 4861 | 60.68 |
| | 20.0 | 5842 | 5821 | 99.64 | 13271 | 7961 | 60.03 |
| | 25.0 | 5685 | 5588 | 98.29 | 15669 | 7967 | 64.06 |
| | 30.0 | 9056 | 8619 | 95.17 | 17550 | 8500 | 48.43 |
| | 35.0 | 11395 | 10437 | 91.59 | 19154 | 4056 | 21.17 |
| 6.0 | 15.0 | 8439.3 | 7137 | 84.57 | 17571.9 | 7013 | 39.91 |
| | 20.0 | 10258 | 9521 | 92.82 | 21967 | 8871 | 40.38 |
| | 25.0 | 14535 | 13549 | 93.21 | 20699 | 8294 | 40.07 |
| | 30.0 | 15958 | 14823 | 92.89 | 23423 | 9547 | 40.76 |
| | 35.0 | 23332 | 19637 | 84.16 | 44253 | 8225 | 18.59 |
| 10.0 | 15.0 | 12389 | 8773 | 70.81 | 23402 | 5351 | 22.87 |
| | 20.0 | 19137 | 17333 | 90.57 | 33173 | 9413 | 28.37 |
| | 25.0 | 23182 | 20219 | 87.21 | 29407 | 9634 | 32.76 |
| | 30.0 | 24342 | 20512 | 84.27 | 43191 | 12471 | 28.87 |
| | 35.0 | 29386 | 21710 | 73.88 | 44016 | 3965 | 9.01 |

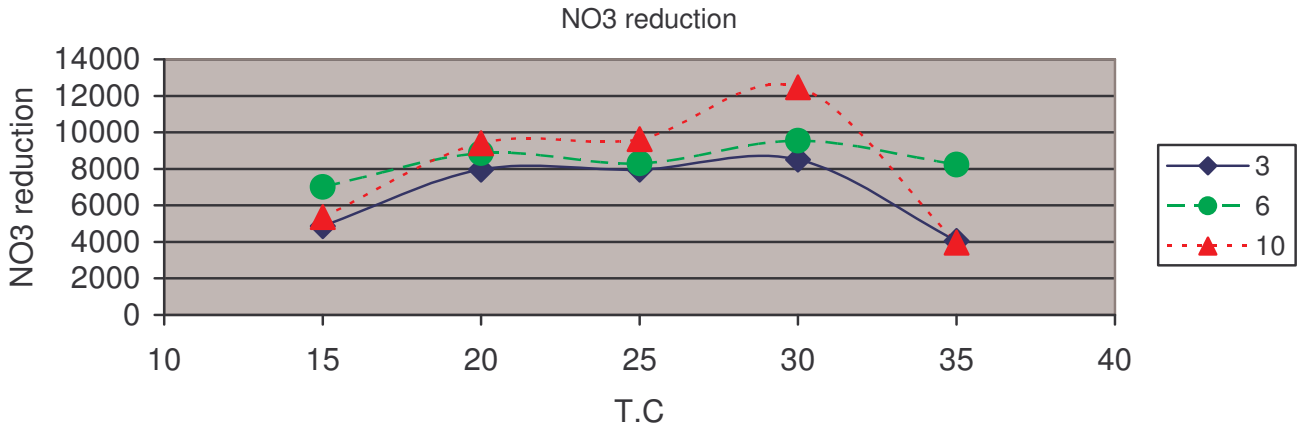


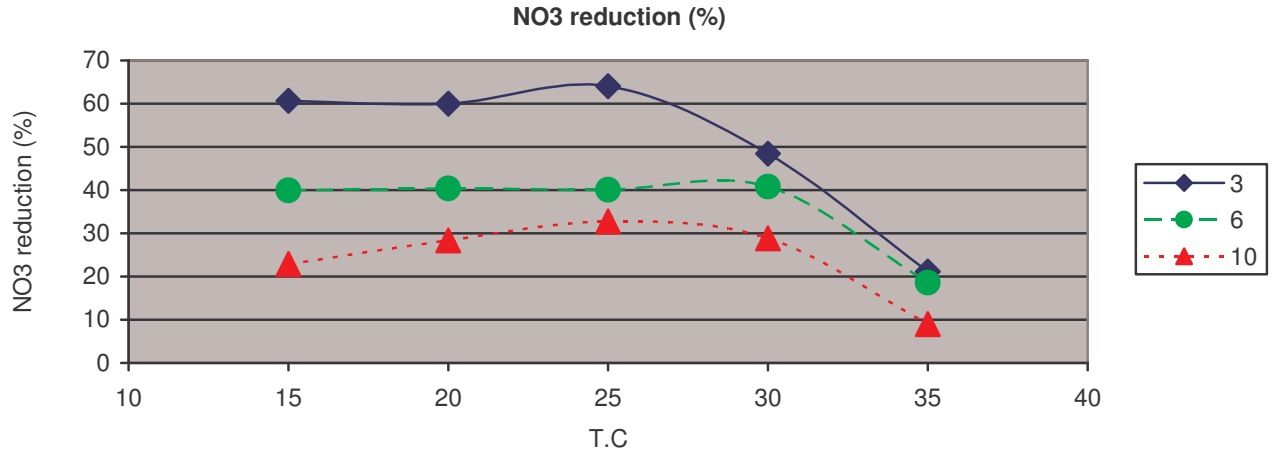




Fig(1) effect of NO3 concentration & temperature on NO3 uptake , reduction & reduction (%) of Corn plant



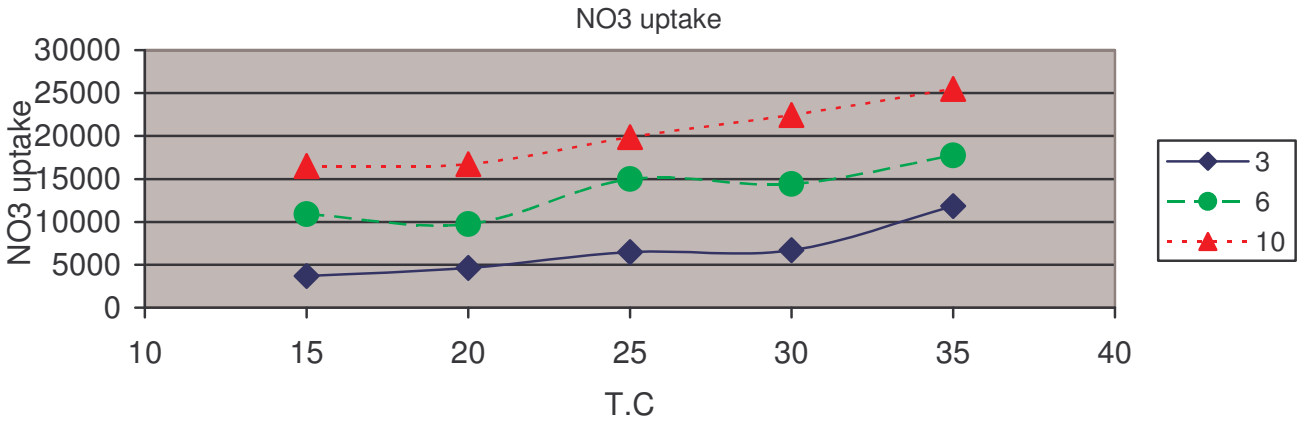



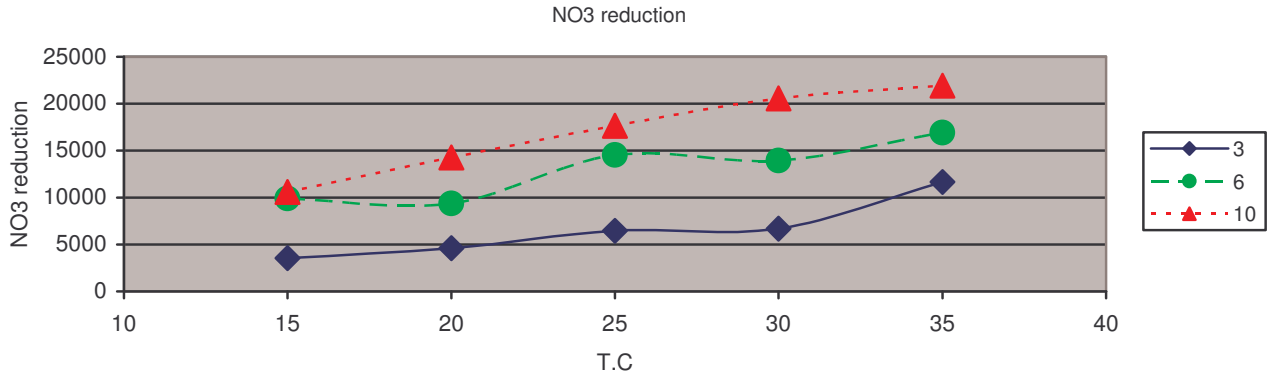



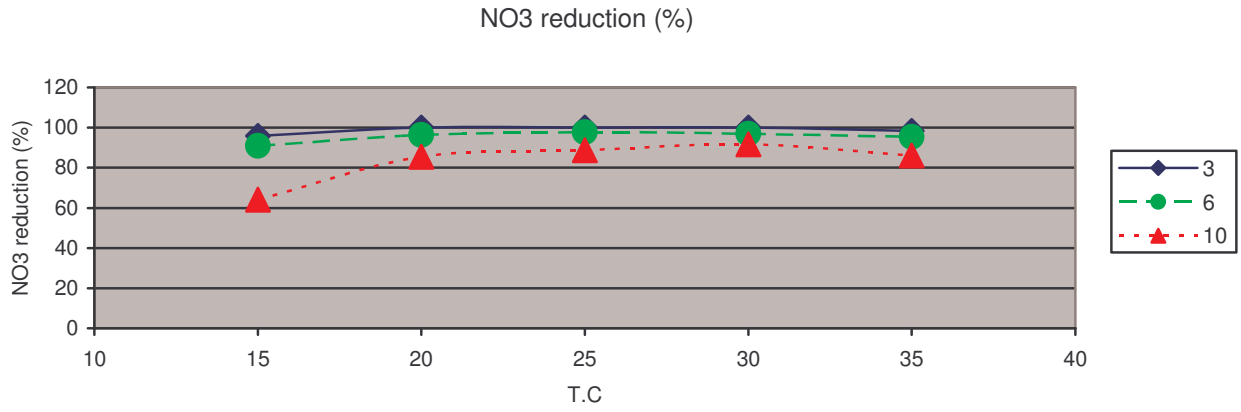

Fig(2) effect of NO3 concentration & temperature on NO3 uptake , reduction & reduction (%) of Barley plant

Table (2) effect of NO3 concentration & temperature & sugar(1%) on NO3 uptake , reduction & reduction (%)

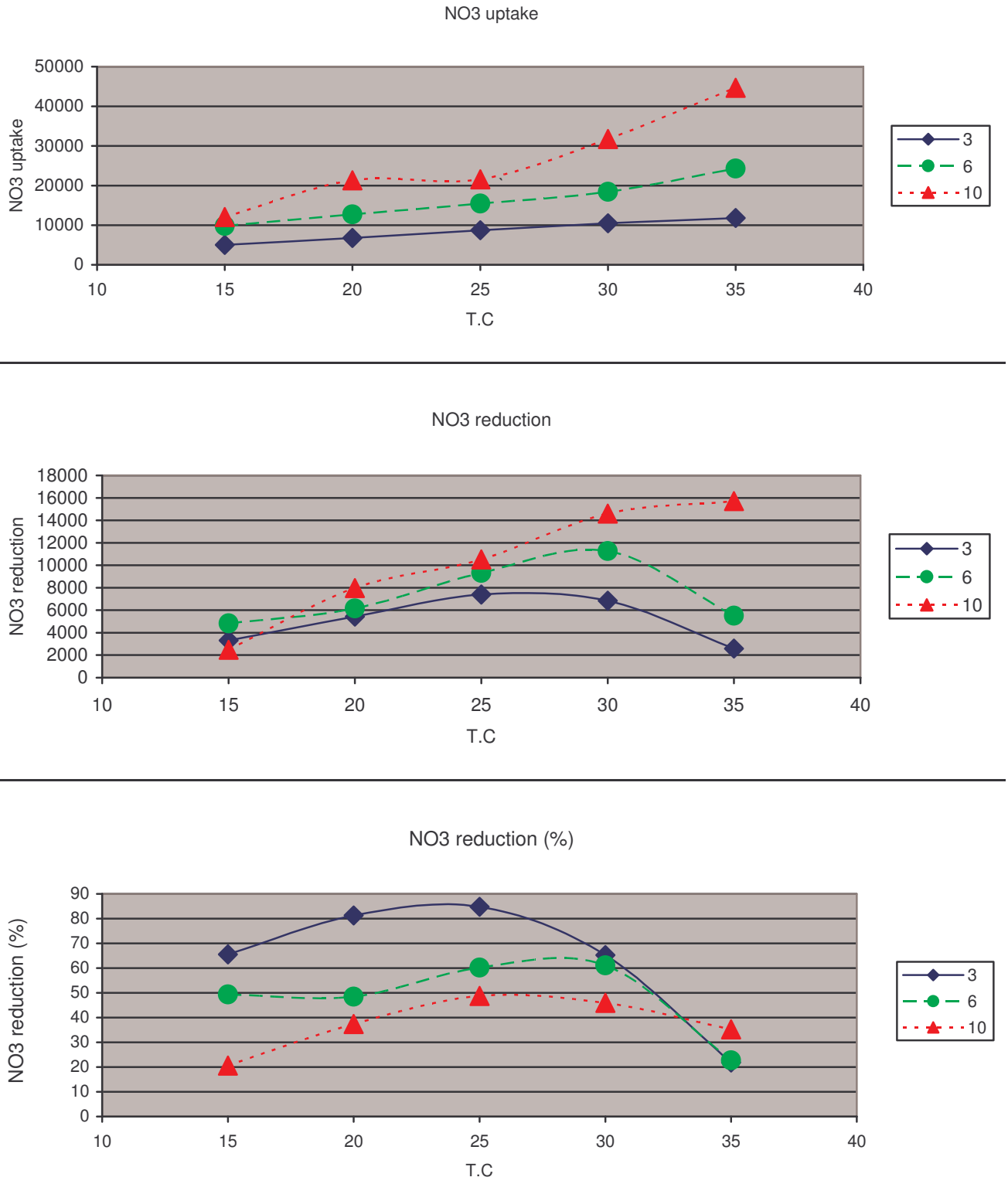
| Suc(%) | NO3.Conc(m. mole) | TC0 | Corn | | | Barley | | |
|---|---|---|---|---|---|---|---|---|
| | | | NO3 uptake uM | NO3 red. | NO3 red. (%) | NO3 uptake uM | NO3 red. | NO3 red. (%) |
| 1.0% | 3.0 | 15.0 | 3680 | 3527 | 95.84 | 5049 | 3307 | 65.50 |
| | | 20.0 | 4617 | 4617 | 100 | 6764 | 5437 | 81.27 |
| | | 25.0 | 6463 | 6463 | 100 | 8734 | 7405 | 84.78 |
| | | 30.0 | 6684 | 6687 | 100.04 | 10474 | 6831 | 65.22 |
| | | 35.0 | 11845 | 11651 | 98.36 | 11796 | 2579 | 21.86 |
| | 6.0 | 15.0 | 10883 | 9894 | 90.91 | 9798 | 4829 | 49.29 |
| | | 20.0 | 9722 | 9356 | 96.24 | 12713 | 6163 | 48.48 |
| | | 25.0 | 14993 | 14637 | 97.63 | 15466 | 9316 | 60.23 |
| | | 30.0 | 14402 | 13952 | 96.88 | 18446 | 11273 | 61.11 |
| | | 35.0 | 17739 | 16917 | 95.37 | 24326 | 5520 | 22.69 |
| | 10.0 | 15.0 | 16518 | 10602 | 64.18 | 12054 | 2476 | 20.54 |
| | | 20.0 | 16724 | 14285 | 85.42 | 21353 | 7982 | 37.38 |
| | | 25.0 | 19880 | 17648 | 88.77 | 21601 | 10522 | 48.71 |
| | | 30.0 | 22430 | 20563 | 91.68 | 31781 | 14591 | 45.91 |
| | | 35.0 | 25478 | 21917 | 86.02 | 44657 | 15722 | 35.21 |




Fig(3) Effect of NO3 concentration , temperature & sugar (1%) on NO3 uptake , reduction & reduction (%) of Corn plant




Fig(4) Effect of NO3 concentration , temperature & sugar (1%) on NO3 uptake , reduction & reduction (%) of Barley plant

## DISSCUSION

Nitrate reduction was essentially idendintical to uptake in response to ambient nitrate. This shows the strong regulatory effect that nitrate uptake has on its subsequent reduction by furnishing the nitrate flux into the plant ( Chantartwong et al ,1976 ). Rate of nitrate assimilation based on the disappearance of nitrate from detached leaves preloaded with nitrate may be confounded by the different nitrate pools found in plants as it is recognized that cell of higher plants have both metabolic ( easily accessible and cytoplasmic ) and storage ( probably vacualar ) pools ( Aslam et al.1976 ) they indicated that after leaves are excised and removed from the roots supplying nitrate, nitrate is depleted in cytoplasmice pools by both reduction and movement into a storage pool ( vacular ). Efflux of nitrate from the vacule back into the aytoplasm could then regulate the rate of nitrate reduction. Finally it mean that the seedling were capable of lowering their steady-state rates of uptake and reduction. It`s clear from data that corn leaves more efficiency than Barley in adaptation to high ambient NO3 concentration .For temperature effect on nitrate reduction percent increased when the temperature raised from 15°C to 25°C in both plants. But increased temperature from 25°C to 30 °C, the reduction percent decrease and big decrease with raising the temperature to 35°C,this maybe inrelation to nitrate reductase activity. (Hallmark & Huffaker ,1978) observed an increase in enzymatic activity occurred from 0°C to 40°C above which decrease occurred, but in our study the harmfull effect of temperature begin at 30°C.Also high temperature affected the photosynthesis by altering the excitation energy distribution by changing the structure of thylakoids ( Berry and Bjorkman,1988 ) and by changing the activity of he calvin cycle and other metabolic processes. Also Maize ( C4) plants more efficience than Barley ( C3) plants in nitrate reduction percent. (Hallmark & Huffaker ,1978) found sudangrass seedling pretreated in nitrate reduced 80 to 100 of nitrate taken up. While for Barley ( Chantarotwong et al 1976) and wheat (Ashley et al 1976 ) reduced about 35% of that taken up,from this data in Maize we concluded that nitrate reduction was approximetly equal to uptake ( reduction percent 90-100%), while in Barley the nitrate uptake bigger than the reduction ( reduction percent not more than 60%). Also it clear from the results that sucrose addition to the nutrient solution improve the efficiency of Barley to reduce NO3 uptake.

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