▼ Journal of Research (Science), Bahauddin Zakariya University, Multan, Pakistan.
Vol.14, No.2, December 2003, pp. 235-240 ISSN 1021-1012

FOLIAR AMINO ACIDS AND PROTEINS OF MAIZE (*ZEA MAYS*) IN RELATION TO LONG-TERM HYPOXIC CONDITIONS UNDER SUPPLEMENTARY NO_3-N

Habib-ur-Rehman Athar and Seema Mahmood
Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.

Abstract: Maize Plants were grown under normal and long term flooding conditions using three different NO_3-N regimes (196, 294 and 392 mg N kg^{-1} soil). Continuous flooding for 21 days had caused significant changes in N containing compounds. A consistent increase in total soluble proteins was observed when external NO_3-N levels were raised in the non-flooded as well as in the flooded pots. Although, continuing hypoxic conditions had not resulted in any significant alteration in free amino acids but supplemented NO_3-N caused an increase in amino acids under aerated conditions. This study revealed that addition of NO_3-N under waterlogged conditions is not advantageous to N metabolism thus causing an incoherent pattern of amino acids and protein accumulation.

Keywords: Amino acids, flooding, long-term hypoxia, nitrate, N-metabolism, protein, *Zea mays*.

INTRODUCTION

Nitrogen status for any crop is crucial as it affects the uptake of nutrients and photosynthetic activity of plants [Kreuzwieser *et al*. 2002]. High organic nitrogen contents in leaves are also essential for CO_2 fixation [Davies *et al*. 2000a, b]. Waterlogging hinders important metabolic processes because under flooded conditions, oxidative respiration is replaced by fermentation, which yields low energy. Disturbance in cellular energy supply mainly affects nutrients uptake causing their deficiency symptoms. Moreover, steady maintenance of N is vital for plant growth and development [Marschner 1995] but flooding may cause a massive impact on N uptake thus affecting whole plant N-metabolism [Drew 1991, Bertani and Reggiani 1991]. Consequently, amino acids and proteins accumulation in plant tissues change greatly [Muller *et al*. 1996]. Accordingly, under flooding stress, the quality of proteins and amino acids as well as activity of related enzymes vary considerably [GeBler *et al*. 1998].

Early findings showed that application of nitrate-N under waterlogged conditions could decrease the harmful effects of flooding [Arnon 1937, Trought and Drew 1981, and Roberts 1988]. By contrast, in rice, Zhang *et al*. [1990] did not reported any ameliorative effect of NO_3-N on the growth of plants under hypoxia. These contrasting reports led us to undertake the present study and we reported N status (proteins and amino acids) in maize plants in relation to flooding and external applications of NO_3-N. The prime objective of the work was to determine whether excessive amounts of NO_3-N could alleviate the deleterious effects of long-term hypoxia and N metabolism in terms of amino acids proteins synthesis in maize plants.

MATERIALS AND METHODS

Zea mays seeds (Line Hicorn 11) were obtained from a local seed supplier. The experiment was conducted at the Botanical Garden, Bahauddin Zakariya University, Multan, Pakistan (30°11 N and 71°28 E), in a green house with natural sunlight. The average day and night temperatures were 31 ± 6°C and 23 ± 4°C, respectively, relative humidity 40.5% and day length 11-12 hrs. The experiment was arranged in a Randomized Complete Block design with six blocks, each block containing two waterlogging treatments and three NO_3-N treatments. About 400 seeds were germinated in plastic Petri dishes. After eight days, 6 seedlings of comparable size were transplanted equidistant in to each mosaic cemented pot (30 cm diameter) containing 15 kg sandy loam soil.

SOIL ANALYSIS

The physico-chemical characteristics of the soil are presented in Table 1. Following Allen *et al*. [1986] exchangeable K, Ca, Mg, Fe, and Mn were estimated in the soil samples (100 g) taken at 0 -10 cm depth from each pot. Soil extracts were made in 1M ammonium acetate solution while waterlogged soil samples were extracted using deoxygenated 1M ammonium acetate solution then were analyzed by an atomic absorption spectrometer (Perkin Elmer, Analyst 100). Nitrate, ammonium-N, and $NaHCO_3$-P were analyzed following Jakson [1958] and Allen *et al.* [1986].

Table1: Physico-chemical characteristics of the original soil (before the addition of NO_3-N) used for the study.

Characteristics	Values
Electrical Conductivity (EC_e) of the soil saturation extract (mS cm^{-1})	2.50 ± 0.10
pH of soil saturation extract	7.12 ± 0.03
Textural class	Sandy Loam
Saturation percentage	27.28 ± 0.21
Nitrate-N (mg N kg^{-1} dry soil)	13.01 ± 1.11
Ammonium-N (mg N kg^{-1} dry soil)	9.54 ± 0.84
$NaHCO_3$-P (mg P kg^{-1} dry soil)	6.04 ± 1.01
Potassium (mg K kg^{-1} dry soil)	178.80 ± 14.20
Calcium (mg Ca kg^{-1} dry soil)	241.40 ± 21.40
Magnesium (mg Mg kg^{-1} dry soil)	181.60 ± 12.40
Iron (mg Fe kg^{-1} dry soil)	38.40 ± 2.46
Manganese (mg Mn kg^{-1} dry soil)	27.90 ± 2.14

The plants were allowed to establish for 34 days (42 days after sowing) before the start of waterlogging treatment and addition of NO_3-N. The soil was maintained at field capacity for control plants while the other pots were flooded to the soil surface (waterlogged). The three NO_3-N concentrations 196, 294 and 392 mg N kg^{-1} soil as KNO_3 were applied at the time of flooding. The K levels in all the treatments were maintained same with KCl. The flooding and NO_3 treatments continued for a period of 21 days when plants were 3 weeks old. Plants were harvested after 42 days then total free amino acids and soluble proteins in the leaves of stressed and non-stressed plants were measured.

SOLUBLE PROTEINS ESTIMATION

Soluble proteins were estimated as described by Lowry *et al.* [1951] and the optical densities of the reaction mixtures were read at 620 nm on a spectrophotometer (Hitachi, U2000).

DETERMINATION OF TOTAL FREE AMINO ACIDS

For the estimation of amino acids, 2% ninhydrin solution was used and the optical densities of the colored solutions were read at 570 nm.

STATISTICAL ANALYSIS

The data for total free amino acids and soluble proteins were subjected to analysis of variance using COSTAT computer package [Cohort Software, Berkeley, California]. Since there was a marked effect of waterlogging on total free amino acids, the data within each waterlogging treatment was also analyzed separately to assess the effect of supplementary nitrate-N. The mean values were compared with least significance difference test following Snedecor and Cochran [1980].

RESULTS AND DISCUSSION

The results of present study (Fig. 1 and Table 2) show that long-term flooding has caused a marked increase in soluble proteins in leaves of *Z. mays.* Protein contents augmented consistently in response to external applications of NO_3-N under both non-flooded and flooded conditions. Longer periods of flooding are assumed to influence N nutrition of plants thus can alter proteins [GeBler *et al.* 1998]. Modified pattern of protein accumulation under flooding and nitrate regimes is in conformity to the early findings of Ashraf and Habib-ur-Rehman [1999].

Table 2: Analysis of variance summaries (mean squares) for total soluble proteins and total free amino acids in maize leaves (42-days old plants) when subjected for 21 days to varying concentrations of NO_3-N under aerated and waterlogged conditions in soil culture.

Source of variation	D.F.	Total soluble proteins	Free amino acids
Nitrogen	2	39.187**	17.865***
Waterlogging	1	222.626***	5.712 ns
Interaction	2	6.150 ns	13.236**

, * Significant 0.01, and 0.001 levels respectively; ns = non-significant

Data presented in Fig. 1 depicted that total free amino acids decreased when plants were grown under non-aerated conditions. However, augmented concentrations of NO_3-N changed this biochemical attribute as a greater proportion of amino acids was observed at the elevated NO_3-N level (392 mg NO_3-N kg^{-1}). Although, free amino acids amplified considerably at 294 mg NO_3-N kg^{-1} under aerated condition but altered pattern of amino acids accretion can be attributed to distressed N uptake under anoxic conditions.

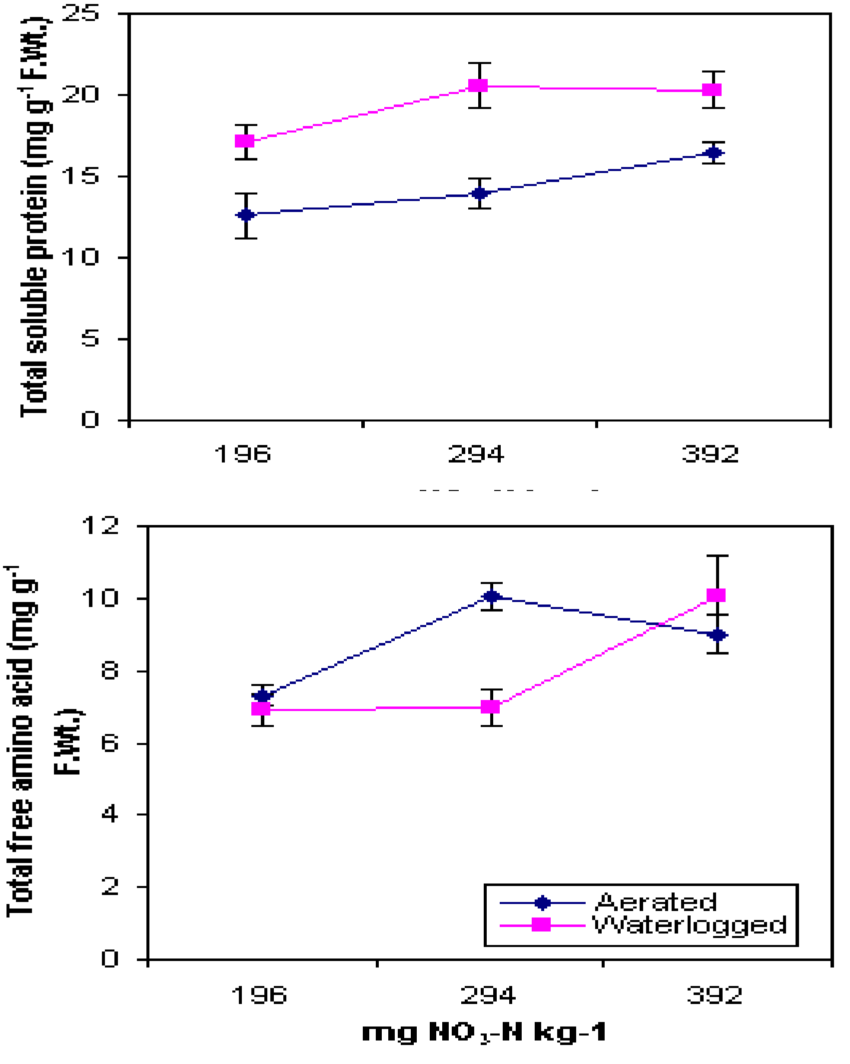

Fig. 1: Total soluble proteins and total free amino acids in maize leaves (42-days old plants) when subjected for 21 days to varying concentrations of NO_3-N under aerated and waterlogged conditions in soil culture [LSD (N x WT) = 2.18 at 5% level of probability]

It has been well reported that replacement of oxidative respiration by alcoholic fermentation under anaerobic conditions hinders the activity of certain enzymes, which are involved in protein synthesis [Good and

Paetkau 1992]. Our results indicated that addition of NO_3-N has resulted in larger amino acids contents, which may accumulate due to decline in protein synthesis [Bertani and Reggiani 1991]. At the same time, maize plants also showed increased concentrations of soluble proteins in leaves under long-term flooding as well as due to addition of NO_3-N. The inconsistency observed here is not surprising and accords with the findings of Ashraf and Mahmood [1990], and Kreuzwieser *et al.* [2002] who observed comparable results when plant species were subjected to longer period of inundation.

The biochemical attributes studied here in relation to flooding and NO_3-N applications have provided some manifestations of altered N metabolism under hypoxia. It can be assumed that reduction of nitrates occurred due to de-nitrification and toxic concentrations of ammonium evolved when plants were exposed to long-term flooding. Thus N uptake under such conditions has greatly been changed which might has caused an alteration of nitrogen containing compound by inducing anoxic metabolism. It can be concluded from the work reported here that external application of NO_3-N did not produced any ameliorating effects on the growth of maize plants under flooding stress.

References

Allen, S.E., Grimshaw, H.M. and Rowland, A.P. (**1986**) “Chemical Analysis”, In: P.D. Moore and S.B. Chapman (Eds.) *Methods in Plant Ecology*, Blackwell Scientific Publications, Oxford, pp. 258-344.

Arnon, D.F. (**1937**) “Ammonium and nitrate nutrition of barley at different seasons in relation to hydrogen ion concentrations, manganese, copper and oxygen supply”, *Soil Sci*., 44, 91-113.

Ashraf, M. and Habib-ur-Rehman (**1999**) “Mineral nutrient status of corn in relation to nitrate and long-term waterlogging”, *J. Plant Nutrition*, 22(8), 1253-1268.

Ashraf, M. and Mahmood, S. (**1990**) “Effects of waterlogging on growth and some physiological parameters of four *Brassica* species”, *Plant and Soil*, 121, 203-209.

Bertani, A. and Reggiani, R. (**1991**) “Anaerobic metabolism in rice roots”, In: M.B. Jackson, D.D. Davies and H. Lambers (Eds.) *Plant Life Under Oxygen Deprivation*, SPB Academic Publishing, The Hague, The Netherlands, pp. 187-200.

Davies, C.L., Turner, D.W., Dracup, M. (**2000a**) “Yellow lupin (*Lupinus luteus*) tolerates waterlogging better than narrow-leafed (*L. angustifolius*): I. Shoot and root growth in a controlled environment”, *Aust. J. Agric. Res*., 51, 701-709.

Davies, C.L., Turner, D.W., Dracup, M. (**2000b**) “Yellow lupin (*Lupinus luteus*) tolerates waterlogging better than narrow-leafed (*L. angustifolius*): II. Leaf gas exchange, plant water status, and nitrogen accumulation”, *Aust. J. Agric. Res*., 51, 711-719.

GeBler A., Schneider S., Weber P., Hanemann U. and Rennenberg, H. (**1998**) "Soluble N compounds in trees exposed to high loads of N: a comparison between the roots of Norway spruce *(Picea abies)* and beech *(Fagus sylvatica)* trees grown under field conditions", *New Phytologist.,* 138, 385-399.

Good, A.G. and Paetkau, D.H. (**1992**) "Identification and characterization of hypoxically induced maize lactate dehydrogenase gene", *Plant Mol. Biol*., 19, 693-697.

Jackson, M.L. (**1958**) "Soil Chemical Analysis", Prentice Hall, Englewood Cliffs, New York.

Kreuzwieser, J., Furniss, S. and Rennenberg, H. (**2002**) "Impact of waterlogging on the N-metabolism of flood tolerant and non-tolerant tree species", *Plant, Cell and Environ*., 25, 1039-1049.

Marschner, H. (**1995**) "Mineral Nutrition of Higher Plants", 2^{nd} ed., Academic Press, London, U.K.

Muller, B., Touraine, B. and Rennenberg, H. (**1996**) "Interaction between atmospheric and pedospheric nitrogen nutrition in spruce *(Picea abies* L. Karst.) seedlings", *Plant Cell and Environ.,* 19**,** 345-355.

Roberts, J.K.M. (**1988**) "Cytoplasmic acidosis and flooding tolerance in crop plants", In: D.D. Hook, W.H. McKee, J. Gregory, V.G. Burrell, M.R. Devoe, R.E. Sojka, S. Gilbert, R. Banks, L.H. Stolzy, C. Brooks, T.D. Mathews and T.H. Shear (Eds.) *The Ecology and Management of Wetland*, Becknham, U.K.

Snedecor, G.W. and Cochran, W.G. (**1980**) "Statistical Methods", 7^{th} ed., Iowa State University Press, Ames, Iowa.

Trought, M.C.T. and Drew, M.C. (**1981**) "Alleviation of injury to young wheat plants in anaerobic solution cultures in relation to the supply of nitrate and other inorganic nutrients", *J. Exp. Bot*., 32, 509-522.

Zhang, B.G., Puard, M. and Couchat, P. (**1990**) "Effect of hypoxia, acidity and nitrate on inorganic nutrition in rice plants", *Plant Physiol. and Biochem*., 28, 655-661.