ACTA AGROBOTANICA
Vol. 57, z. 1-2 – 2004
s. 41–48

Nitrate reductase activity in cabbage (*Brassica oleracae* var. *capitata*) seedlings affected by the different nitrogen fertilizer forms

Metin TURAN[1*], Yildrum SEZEN

[1] Ataturk University, Faculty of Agriculture, Department of Soil Science, Erzurum/Turkey
* Corresponding Author: mturan@atauni.edu.tr

(Received: 23.04.2003)

Summary

The effect of different nitrogen fertilizer (potassium nitrate, ammonium nitrate, ammonium sulphate, urea and farmyard manure) on nitrate reductase activity in cabbage (*Brassica oleracea* var. *capitata*) seedlings were studied. pH of the plant growth media was higher in the nitrate fertilizer treatment than the ammonium and other fertilizer forms. NO_3^--N application increased NRA in plant, but NH_4^+ -N decreased NRA in plant. Harvesting date and different fertilizer doses increased NRA while NH_4^+ -N decreased plant nitrate uptake. There was a significant relationship between NRA and fertilizer types.

Key Words: Nitrate reductase, nitrate, cabbage, fertilizers

INTRODUCTION

The ever-growing world population constantly imposes the need for increasing food supply. Carbon and nitrogen are major constituents of plant biomass. Their availability in the environment and their incorporation into cellule components are major factor in plant growth and biomass production (Foyer and Ferrario, 1994). Nitrate is the most favourable nitrogen sources for plants (Crawford, 1994). The reduction of nitrate to nitrite carried out by the enzyme nitrate reductase (NR) has been claimed to be rate-limiting step in the assimilation of nitrate nitrogen by plants (Crawford, 1994). Providing plants with excess nitrogen fertilizer, a common agricultural practice, results in nitrate accumulation in the plant on the one hand and its leaching into ground water on the other, both having undesired environmental effects.

There is general agreement that nitrate enhances nitrate reductase activity (NRA). In most cases, NH_4^+ and amino acids have reverse effect. The influence of these metabolites, however, is not an equivocal. Srivastava (1980) quotes examples in which NH_4^+ did not depress NRA. Radin (1975) reported that at pH 5 the inhibiting effect of NH_4^+ on NRA was less than at pH 7. In his experiment, the addition of amino acids depressed the induction of NRA in roots but not in leaves of *Gossypium hirsutum*.

To study the effect of various metabolites on NRA, it is appropriate to take into consideration of these metabolites in plant tissue. Robin et al. (1979) in studying the effect of nitrate on NRA in roots and shoots of maize seedlings, found a clear relationship between nitrate concentration and the NRA in the tissue. In leaves, the relationships was characterized by a saturation type curve, while in the roots was sigmoidal.

The objectives of this study were to determine the effects of different nitrogen sources such as NO_3^- and NH_4^+ and nitrogen levels on plant nitrate uptake and nitrate reductase activity in cabbage (*Brassica oleracea* var. *capitata*) seedlings.

MATERIALS AND METHODS

Experimental design: the experiments were laid out as randomised block design with four rates of N application (0, 100, 200 and 400 kg N ha^{-1}), five nitrogen sources [potassium nitrate, ammonium nitrate, urea, ammonium sulphate and farmyard manure (FYM)], 3 replicates cabbage (*Brassica oleracea* var. *capitata*) plants. Triple super phosphate (120 kg P_2O_5 ha^{-1}) and potassium sulphate (150 kg K_2O ha^{-1}) were applied as basal fertilizer.

Plant cultivation and sampling: seeds of cabbage (*Brassica oleracea* var. *capitata*) were moisted in perlite media. Plants were grown in a growth chamber at 28°C day temperature and 10°C at night with irrigation 42 wm^{-2}. Day length was roughly 14 h during the experimental period.

Experimental period: each morning, pH and N were determined in the samples of growing media to calculate nitrate and NH_4^+ - N uptake by seedling during the experiment period. Uniform seedlings were harvested in the morning on 3, 9, 18 and 27 d after exposure of seedlings to different nitrogen fertilizer forms. The plants were thoroughly washed with distilled water and excess water was removed by using filter paper. Four samples per treatment were taken to determination of NRA, 500 to 1000 mg of fresh samples weight were stored in –30°C. Similar single samples were taken for determination of nitrate and NH_4^+- N. For the determination of NO_3^-, 1 g dried and ground samples of dry-matter were analysed using the method described by Havlin and Soltanpour (1980). Ammonium was assayed on amino acid analyse (ion exchange procedure with lithium citrate buffers) (Van Beusichem and Neeteson, 1982). Nitrate reductase was assayed by following the conversion of NO_3^- to NO_2^- after Barro et al. (1994) in which reduced monotlavin nucleotide is the reducing agent. Nitrite was determined by adding 1 ml of (W/V) sulphanilamide in 1 N HCl and 1 ml of 0.2% (W/V) N-(1-naphthyl) – ethylenidamine dihydrochloride. The absorbance was read at 540 nm, after 15 min.

Statistical analysis: each pot was considered as replicate and all of the treatments were repeated three times. Regression analysis was performed using Statistical program. The means are reported.

RESULTS

The pH changes in the growing media throughout the experimental period are shown Table 1. pH of the growing media was higher in the NO_3^- fertilizer treatment than the NH_4^+ and the other fertilizer form. pH was increased with increasing the fertilizer doses and time after exposure to potassium nitrate, ammonium nitrate, urea and FYM, while decreased in ammonium sulphate fertilizer. A greater uptake of cations including NH_4^+ is associated with the H efflux characteristic of NH_4^+ - N nutrition. Where as the greater uptake of anions that usually occurs in NO_3^- fed plant is counterbalanced by an equivalent net OH efflux (Van Beusichem, 1981; Van Beusichem and Neetson, 1982).

Plants grew vigorously and there were no major differences in appearance and growth rate of plants between treatments. But NRA was significantly lower in the NH_4^+-N treatment as compared with the other treatment (Figure 1). The effect of N forms on NRA followed the well known fact that NO_3^- has a stimulating influence, and NH_4^+-N and organic acid have a depressive influence on NRA. At all harvesting dates, there was a clear influence of the type of N nutrition on the NRA. These were the highest in the nitrate treatments such as KNO_3^-, NH_4NO_3, while those of the NH_4^+-N treatment were extremely low (Figure 1). Fertilizer doses significantly affected plant NRA and increased NRA with increasing fertilizer doses in all treatments while NH_4^+-N decreased NRA (Figure 1). The highest NRA was found in the KNO_3^- treatment with 400 kg ha^{-1} N. Throughout the experimental period, the NRA of the urea and FYM treatment did not show any significant differences. The low NRA in the 400 kg ha^{-1} N of the ammonium sulphate treatment are consistent with the earlier reports that NH_4^+-N depress NRA while nitrate has an increasing effect (Radin, 1975, Oaks et al., 1977).

Harvesting date, fertilizer types and fertilizer doses also affected plant nitrate uptake (Figure 2). During the first period of the experiment (1-3 d after exposure to different N forms), nitrate uptake in the KNO_3^- treatments were about equal for all of the treatment. In the following period (4-9 d) the differences in the nitrate uptake in the KNO_3^- treatments were about 88.8%, 91.8% and 93.7% higher in the 100, 200, 400 kg ha^{-1} N doses than controls, respectively. During the last period (19-27 d), nitrate uptake rates in the KNO_3^- treatments were about 81.8%, 81.2% and 87.6% in the 100, 200, 400 kg ha^{-1} N doses, respectively. The same pattern was the true for the NRA in the all of the treatments except for ammonium sulphate treatment (Figure 1). Lower difference in NRA between both treatments was found at the beginning, higher at the end of the experimental period. Fertilizer doses as increased and exposure time as increased; NRA of plants was increased KNO_3, NH_4NO_3, urea and FYM, decreased $(NH_4)_2SO_4$ treatment. It should be stressed that the nitrate uptake rates of the $(NH_4)_2SO_4$ by seedlings increased very little during the experimental period, while nitrate uptake rates of the nitrate treated plants clearly increased. The same is true for the NRA.

Table 1. pH values and NH_4^+- N content of cabbage plant in different fertilizer treatment throughout the experimental period.

N doses kg ha^{-1}		100		200		400	
Fertilizer type	Time after exposure to N fertilizer, day	pH	NH_4 me g fresh wt^{-1}	pH	NH_4 me g fresh wt^{-1}	pH	NH_4 me g fresh wt^{-1}
KNO_3	3	7.06 b	0.90 g	7.10 b	0.92 f	7.23 b	1.12 d
	9	7.11 b	1.22 g	7.16 b	1.31 f	7.33 ab	2.55 d
	18	7.24 b	2.35 g	7.36 ab	2.50 e	7.45 ab	2.86 d
	27	7.50 a	3.87 ef	7.68 a	3.90 e	7.85 a	4.15 d
NH_4NO_3	3	7.05 c	0.74 g	7.08 c	1.22 e	7.10 b	2.42 d
	9	7.09 c	5.35 e	7.14 b	9.70 d	7.20 b	18.20 c
	18	7.14 b	17.41 c	7.34 ab	23.66 b	7.45 ab	46.83 b
	27	7.38 ab	22.66 c	7.50 a	35.40 ab	7.53 a	57.66 ab
Urea	3	7.08 c	0.82 g	6.85 c	0.90 f	6.80 c	1.02 d
	9	6.80 c	3.41 ef	6.70 c	3.63 e	6.60 d	3.88 d
	18	7.10 b	6.83 e	6.90 c	6.96 d	7.00 c	7.20 d
	27	7.20 b	8.36 e	6.95 c	8.74 d	7.10 c	9.06 cd
$(NH_4)_2SO_4$	3	6.90 c	0.84 g	6.40 d	0.91 f	6.40 c	1.60 d
	9	6.70 c	36.85 b	6.10 de	45.40 ab	5.60 de	63.22 ab
	18	6.00 c	46.21 a	5.30 e	53.80 a	5.10 de	88.63 a
	27	5.70 d	53.83 a	5.10 e	66.75 a	4.80 e	93.12 a
FYM	3	7.00 c	0.90 g	7.00 c	0.9 f	7.10 b	1.05 d
	9	7.10 b	5.22 e	7.13 b	5.55 d	7.25 b	5.66 d
	18	7.15 b	9.44 d	7.18 b	10.33 c	7.35 ab	13.44 c
	27	7.15 b	11.66 d	7.20 b	12.41 c	7.60 ab	15.33 c

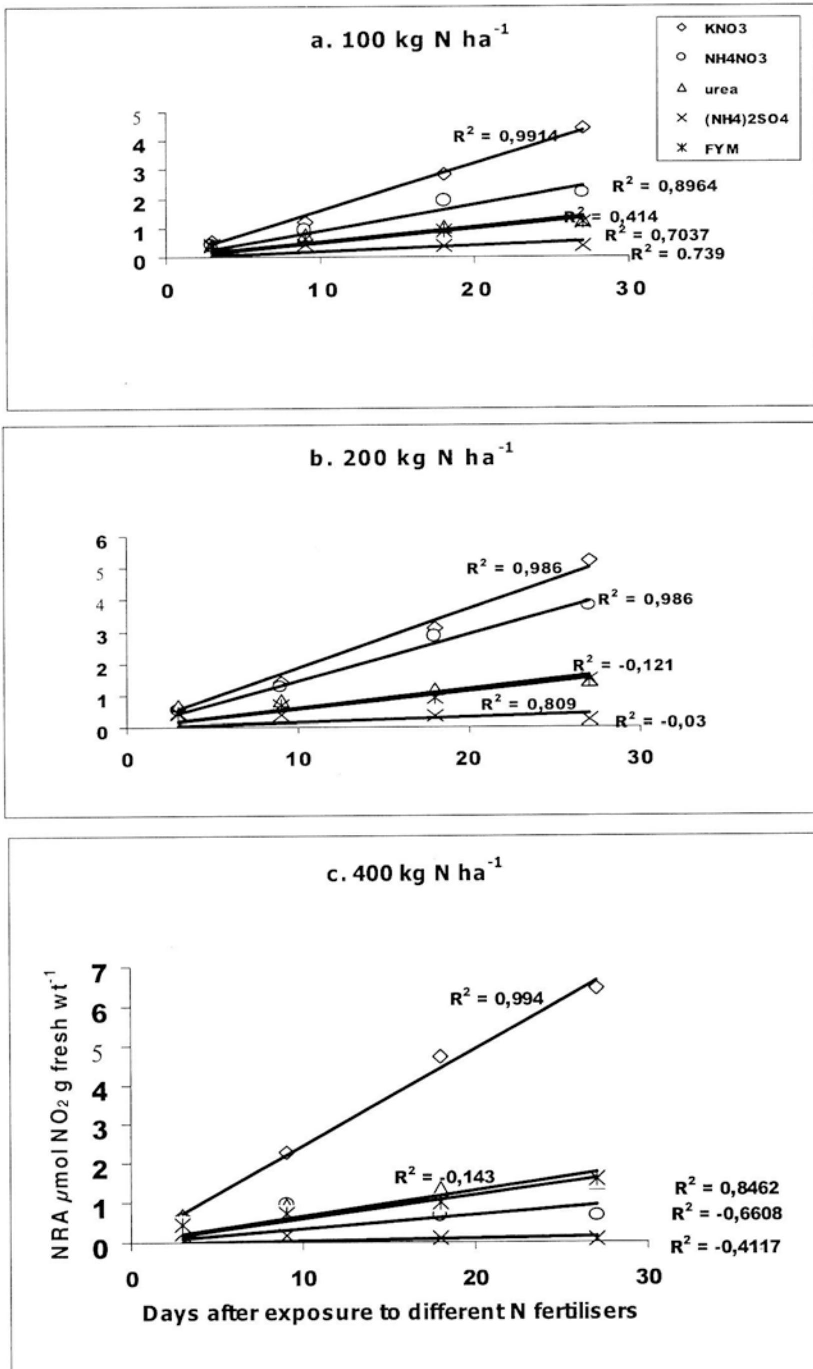

Figure 1. Nitrate reductase activities of various day – old seedlings following different N fertilizer application

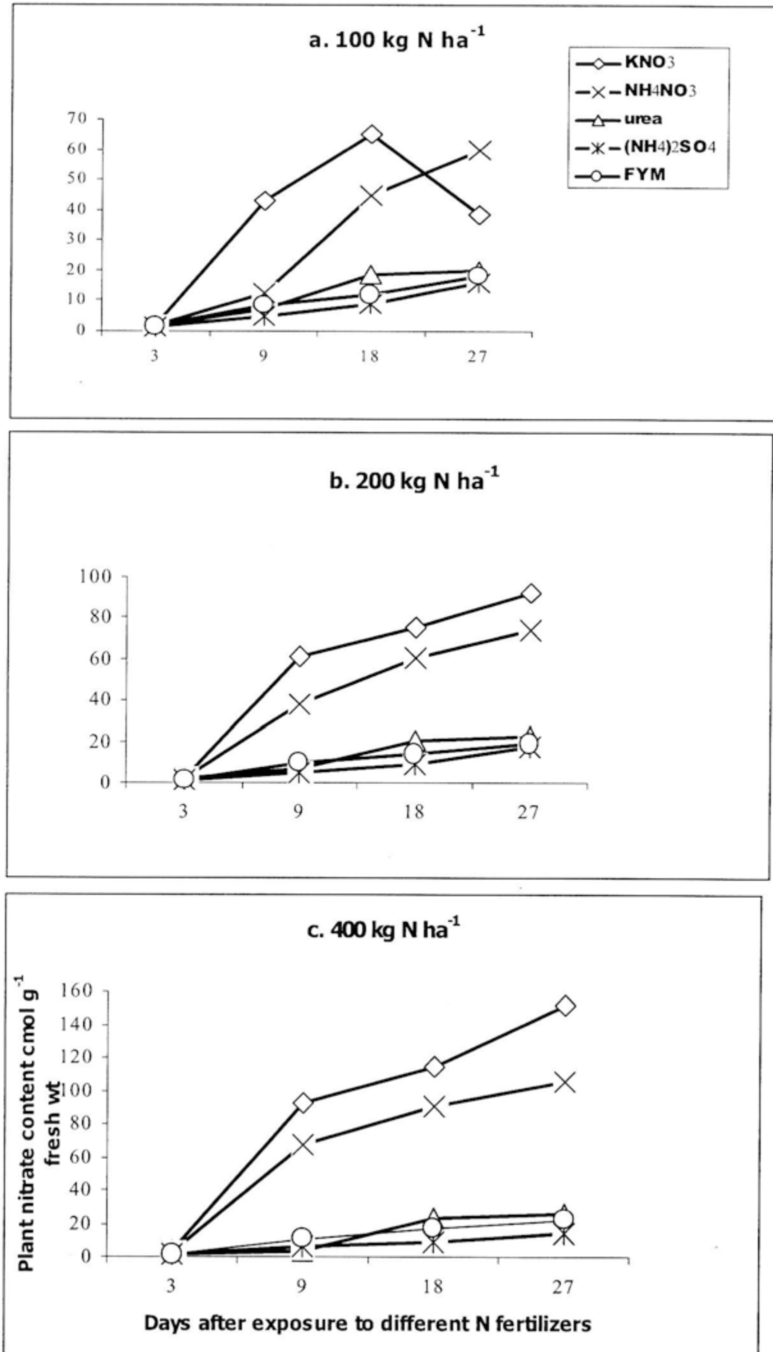

Figure 2. Plant nitrate contents activities of various day – old seedlings following different N fertilizer application.

There was significant relationship between plant NRA and fertilizer type (Figure 1). The highest regression was the KNO_3 fertilizer type and NRA (r^2=0.99). These findings support to the idea that nitrate turnover. Alcalinization effect of nitrate reduction is clearly reflected by the pH of the growth media. Here, the lower nitrate uptake rates of 1-3 d are reflected by a less steep pH increase (7.2) than the higher uptake rates of the last days with an pH increase to 7.85 in KNO_3^- treatment.

DISCUSSION

In the cell, the pH increase may be buffered by an enhanced production of malate (Hiatt and Leggett, 1974; Smith and Raven, 1979). Despite this buffer power, nitrate reduction may increase the pH of the cytoplasm to some degree. Thus, Kikby and Mengel (1967) and also Blevins et al. (1974) found a pH increase in the press sap of nitrate fed plants. It is feasible that this pH increase, or processes related to this increase such as the synthesis of malate or other organic anions, has a promoting effect on NRA.

The hypothesis of nitrate reduction rather than the presence of nitrate or/and ammonium and amino acids with influence NRA is also consistent with the results of other researchers and might explain some controversy in the literature. Thus, the effect of NH_4^+-N on NRA is not unequivocal (Srivastava, 1980; Guerrero et al., 1981). In light of the hypothesis presented here, NH_4^+-N as well as the various amino acids only affected NRA, if these metabolites influence nitrate reduction. This is generally the case, and NH_4^+-N as well as amino acids are known to depress nitrate uptake, nitrate reduction, and the metabolism of nitrate N (Oaks et al., 1977; Weissman, 1972).

The results of Robin et al. (1979) are also consistent with the hypothesis presented above. The workers found that increasing NO_3^- content in cabbage seedling were not paralleled by linear increasing of NRA, but the curves for NRA levelled of at certain NO_3^- concentration in plants. The findings of Radin (1975) that the depressive effect of NH_4^+ on NRA could be alleviated by extremely high NO_3^- concentration is compatible with the hypothesis that nitrate reduction itself regulates NRA.

Our results indicated that fertilizer type, fertilizer doses and plant growth periods affected on the plant nitrate uptake and NRA. Application of the nitrate fertilizers enhanced plant nitrate uptake and plant NRA, but ammonium fertilizers decreased.

REFERENCES

Barro F., Fontes A. G., Maldonado J. M., 1994. Nitrate uptake reduction by durum wheat (*Triticum turgidum*) and Tritordeum (*Hordeum chilense x Triticum turgidum*). Plant Physiol., 143, 313.

Blevins D. G., Hiatt A. J., Lowe R. H., 1974. The influence of nitrate and chloride uptake on expressed sap pH, organic acid synthesis, and potassium accumulation in higher plants. Plant Physiol 54: 82-87.

Crawford N. M., 1994. Nitrate, phosphate, and iron assimilation in *Arabidopsis*. In: E. M. Meyerowitz, C. R. Somerville (Eds.), *Arabidopsis* Laboratory Press, Col Spring Habor, pp.111-146.

Foyer C. H., Ferrario S. F., 1994. Modulation of carbon and nitrogen metabolism in transgenic plant with a view to improve biomass production. Biochem. Soc. Trans. 22: 909-915.

Guerrero M. G., Vega J. M., Losada M., 1981. The assimilatory nitrate reducing system and its regulation. Annu. Rev. Plant Physiol. 32:139-168.

Havlin J. L., Soltanpour P. M., 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Communication in Soil Science and Plant Analysis, 11: 969-98.

Hiatt A. J., Legget J. E., 1974. Ionic interactions and antagonism in plants. In: E. W. Carson, ed. The Plant Root and Its Environment. University Press of Virginia, Charlottesville, pp. 101-134.

Kirkby E. A., Mengel K., 1967. Ionic balance in different tissues of tomato plant in relation to nitrate, urea or ammonium nutrition. Plant Physiol. 42: 6-14.

Oaks A., Aslam M., Boesel I., 1977. Ammonium and amino acids as regulators of nitrate reductase in corn roots. Plant Physiol. 59: 391-394.

Radin J. W., 1975. Differential regulation of nitrate reductase induction in roots and in shoots of cotton plants. Plant Physiol. 55: 178-182.

Robin P., 1979. Etudes de quelques conditions d'extraction de nitrate reductase des racines et plants. Plant Physiol. 55: 178-182.

Robin P., Blayac D., Salsac L., 1979. Influence de l'alimentation nitrique sur la teneur en nitrite et l'activite nitrate reductase des raciness et de feuilles de plantues de Mais. Physiol. Veg. 17: 55-66.

Smith F. A., Raven J. A., 1979. Intracellular pH and its regulation. Annu. Rev. Plant Physiol. 30: 289-311.

Srivastava H. S., 1980. Regulation of nitrate reductase activity in higher plants. Phytochemistry 19: 725-733.

Weissman G., 1972. Influence of ammonium and nitrate nutrition on enzymatic activity in soybean and sunflower. Plant Physiol. 49: 138-141.

Van Beusichem M. L., 1981. Nutrient absorption by pea during dinitrogen fixation. 1. Comparison with nitrate nutrition. Neth. J. Agric. Sci. 29: 259-272.

Van Beusichem M. L., Neeteson J. J., 1982. Urea nutrition of young maize and sugar-beet plants with emphasis on ionic balance and vascular transport of nitrogenous compounds. Neth. J. Agric. Sci. 30: 317-330.

Streszczenie

Aktywność reduktazy azotanowej w siewkach kapusty (*Brassica oleracea* var. *capitata*) w zależności od rodzaju nawozu azotowego

Badano wpływ różnych rodzajów nawozów azotowych (azotan potasu, azotan amonu, siarczan amonu i mocznik) oraz obornika na aktywność reduktazy azotanowej w siewkach kapusty głowiastej (*Brassica oleracea* var. *capitata*). Stosowanie nawozów azotanowych zwiększało aktywność enzymu w roślinach, podczas gdy użycie nawozów amonowych – obniżało. Zależność między aktywnością reduktazy azotanowej i rodzajami nawozów azotowych była istotna.