

BRIEF COMMUNICATION

Response of eggplant to nitrogen supply: molybdenum-nitrate relationships

G. VÍLLORA*, D.A. MORENO and L. ROMERO

*Department of Plant Biology, Faculty of Sciences, University of Granada,
Av. Fuentenueva s/n, E-18071 Granada, Spain*

Abstract

Greenhouse experiments were conducted in two years (1993-1994) with eggplants supplied with 1, 2 or 4 mM NH_4NO_3 as the N source in order to determine its influence on molybdenum (Mo) and nitrate (NO_3^-) content in leaf blades, petioles, and fruits as well as leaf nitrate reductase (NR) activity. The results reveal that 2 and 4 mM NH_4NO_3 altered shoot Mo distribution and thus affecting the NR activity.

Additional key words: *Solanum melongena*, nitrate reductase.

The requirements of plants for molybdenum are lower than for any of the other micronutrients, except nickel. The importance of Mo for plants has long been known and Mo-containing enzymes catalyse NO_3^- -reduction, N_2 fixation and, presumably sulphur reduction (Marschner 1995, Zimmer and Mendel 1999). Plant N metabolism was affected by Mo directly acting on the key-enzyme nitrate reductase (NR) activity, which was used as sensitive bioindicator for the Mo and N status in plants (Lavon and Goldschmidt 1999). Thus, the NR activity can be altered by changing the NO_3^- concentration (Redingbaugh and Campbell 1991, Crawford 1995) or Mo status (Sagi *et al.* 1997). With soil-applied NO_3^- as the N source, the NO_3^- uptake and root-to-shoot translocation rate increase, as does the NR activity (Maynard *et al.* 1976), and Mo-deficiency symptoms could be reduced with respect to the NH_4^+ supply (Chiallou *et al.* 1986). Nevertheless, depending on the plant species and N source, the critical deficiency level of foliar Mo vary between 0.1 and 10 $\mu\text{g g}^{-1}$ (d.m.) (Marschner 1995).

Given that the changes in the N supply could alter Mo content in plant tissues and *vice versa*, Mo content thus could be a function of the N source applied. Therefore, the aim of present study was to test the hypothesis that

eggplant presents a contrasting pattern of Mo and NO_3^- distribution within leaves and fruits as well as leaf nitrate reductase activity in response to increasing N supply.

Eggplants (*Solanum melongena* L. cv. Bonica) were grown in greenhouse in Almería (Spain), for 12-week in 1993 and 1994 under controlled conditions: relative humidity of 60 - 80 %, day/night temperature 30/25 °C, 14-h photoperiod, and irradiance of 400 $\mu\text{mol s}^{-1} \text{m}^{-2}$, measured at the plant apex with a 190 SB quantum sensor (LI-COR, Lincoln, USA). The plant received a nutrient solution (pH 5.5 to 6.0) containing: 1 mM NH_4NO_3 , 1 mM H_3PO_4 , 0.5 mM K_2SO_4 , 0.5 mg dm^{-3} Fe-EDTA, 0.1 mg dm^{-3} $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.05 mg dm^{-3} $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.1 mg dm^{-3} $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, 0.03 mg dm^{-3} $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4 \text{H}_2\text{O}$ and 0.1 mg dm^{-3} H_3BO_3 . The nutrient were supplied gradually over the entire growth period. At the 30-d-old stage, the plants were supplied with NH_4NO_3 at three concentrations (1, 2 and 4 mM). The experiment were arranged in a complete randomized block design with three treatments replicated four times (12 plots) and two plants per pot.

The plants were sampled throughout fruit ripening, from 45-d-old plants to the 90-d-old plants, by collecting 2 leaves per plant from the central part of the stem, at the mature stage. Eggplant fruits (4 fruits per replication)

Received 6 August 2001, accepted 4 December 2001.

Abbreviation: NR - nitrate reductase.

*Corresponding author; fax: (+34) 958 248 995, e-mail: lromero@ugr.es

were harvested at maturity, with acceptable colour, caliber, and firmness. At each sampling, we separated the plant material into two subsamples, one for fresh mass and the other for dried mass, to be stored in plastic bags for the further analyses.

For molybdenum determination dried and ground mass samples (0.15 g leaf blades, petioles, and fruits) were digested in 5 cm³ concentrated HNO₃ in presence of 30 % H₂O₂ (Jones 1991). Next, 0.005 cm³ 0.1 % Pd(NO₃)₂ and 0.005 cm³ Mg(NO₃)₂ were added as a protecting solution to 0.02 cm³ aliquot extraction (as recommended by *Perkin-Elmer*). The reaction mixture was placed in the autosampler (*Perkin-Elmer AS-90*, Boston, USA) and injected into a flame atomic absorption spectrophotometer (*Perkin-Elmer 5100 ZL ZEEMAN*).

For nitrate determination an aqueous extraction of dried and ground material (0.20 g leaf blades, 0.25 g petioles and fruits) in 20 cm³ of *Millipore* water was carried out by continuous shaking for 120 min following centrifugation at 1 000 g. A 0.1 cm³ aliquot was taken for nitrate determination by adding 0.4 cm³ 5 % (m/v) salicylic acid in 6 M H₂SO₄ and 9.5 cm³ 2 M NaOH, and absorbance was measured at 410 nm by spectrophotometry (Cataldo *et al.* 1975).

In vivo NR activity (E.C. 1.6.6.1) was determined by the Jaworski (1971) assay. Leaf blades were cut into 5 mm sections (100 mg) and placed in 10 cm³ of incubation buffer [100 mM K-phosphate buffer, pH 7.5,

and 1 % (v/v) propanol]. The samples were infiltrated and the intracellular spaces of the tissues were flushed with buffer, using a vacuum (0.08 MPa). After 5 min, the vacuum was released and the samples were re-evacuated, then incubated at 30 °C in darkness for 1 h, and finally placed in a boiling water bath to stop the NR activity. The resulting nitrite concentration was determined spectrophotometrically at 540 nm in a reaction mixture containing 2 cm³ of extract, 2 cm³ of 1 % (m/v) sulfanilamide in 1.5 M HCl and 2 cm³ 0.02 % (m/v) N-(1-naphthyl)-ethylenediamine dihydrochloride in 0.2 M HCl (Snell and Snell 1949). The NR activity induced by NO₃⁻ (NR+NO₃⁻) was determined following the same method only using a modified incubation buffer, containing 50 mM KNO₃. The NR induced by Mo (NR+Mo) and NR induced by NO₃⁻ and Mo (NR+NO₃⁻+Mo) were also determined using a modification of the incubation buffer containing 20 mM Na₂MoO₄ and 50 mM KNO₃ plus 20 mM Na₂MoO₄, respectively. The resulting nitrite concentration was also determined spectrophotometrically.

Standard analysis of variance was used for the results as there were no climatic effects on the crop, *ANOVA* being performed on data pooled from both years.

The increased N application significantly ($P < 0.01$) lowered Mo content in leaf blades, with the highest concentration being registered at 1 mM N (Table 1). These results agree with those reported for other crops,

Table 1. Effect of NH₄NO₃ dosage on Mo [$\mu\text{g g}^{-1}(\text{d.m.})$], and NO₃⁻ content [$\text{mg g}^{-1}(\text{d.m.})$] in leaves, petioles and fruits of eggplants and on *in vivo* initial NR activity and on the NR activity in presence of 50 mM NO₃⁻ (NR+NO₃⁻), 20 mM Mo (NR+Mo) and 50 mM NO₃⁻ and 20 mM Mo (NR+NO₃⁻+Mo) [$\text{nmol}(\text{nitrite}) \text{g}^{-1}(\text{f.m.}) \text{s}^{-1}$] in leaves of eggplants. The results of Duncan's multiple range test is represented with lowercase letters. ***, **, * - significant differences at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

NH ₄ NO ₃ [mM]	Leaves Mo	NO ₃ ⁻	Petioles Mo	NO ₃ ⁻	Fruits Mo	NO ₃ ⁻	NR activity			
							initial NR	NR+NO ₃ ⁻	NR+Mo	NR+NO ₃ ⁻ +Mo
1	2.1a	7.82b	2.0a	6.92b	0.5b	9.35ab	1.52a	1.13a	1.03b	2.11a
2	1.2b	12.03a	0.6b	8.15ab	1.8a	8.49b	0.48b	1.01a	1.95a	2.82a
4	1.2b	12.90a	0.6b	10.60a	1.5a	9.89a	0.48b	0.75b	2.07a	2.82a
	**	*	***	*	**	*	***	**	*	ns
LSD _{0.05}	0.4	3.69	0.3	2.78	0.5	0.89	0.40	0.23	0.29	1.05

such as wheat, in which the Mo foliar content in plants supplied with NH₄NO₃ at 0.05 g(N) kg⁻¹(soil) was greater than when the supply reached 0.2 g(N) kg⁻¹(soil) (Wang *et al.* 1999). Meanwhile, the increasing N rates significantly ($P < 0.05$) boosted the NO₃⁻ content in the leaf blades (Table 1), suggesting that the application of NH₄NO₃ raised the content of NO₃⁻ within the plant to a point that exceeded the capacity of NR, glutamine synthase, and glutamine synthetase to reduce NO₃⁻ to NH₄⁺ and NH₄⁺ to amino acids (Andersen *et al.* 1999), resulting in excessive NO₃⁻ accumulation in leaves.

The Mo concentration in the petioles responded to the

N supply, with lower Mo concentrations ($P < 0.001$) at 2 and 4 mM N (Table 1), whereas the NO₃⁻ content in the petioles (Table 1), used as a rapid estimation of plant N status (Andersen *et al.* 1999), showed the highest concentration at 4 mM N, reflecting higher N nutrition.

In fruits, the higher N supply significantly augmented the Mo content ($P < 0.01$; Table 1). As in the case of Mo, the NO₃⁻ content in fruits significantly increased with N dosage applied not only with respect to the high values found in the petiole, but also to excess NO₃⁻ accumulation in storage sites (Gniazdowska *et al.* 1999), which were the fruits in this case (Villora *et al.* 1998). Thus, NO₃⁻

was apparently translocated to the storage sites as a physiological response to an excess of N application.

The N rates applied significantly affected ($P < 0.001$) the initial NR, with the 1 mM rate inducing the highest activity (Table 1), whereas the reactivated NR+NO₃⁻ assay increased at 1 and 2 mM ($P < 0.01$; Table 1). This fact appeared to be related to a increased NO₃⁻ uptake at low soil N content (Pulgar *et al.* 2000), because greater the N availability in soil from the increasing N dosages diminished NR activity. In addition, the fall of initial NR activity at 2 and 4 mM N could be related to the lower foliar content of Mo in these treatments (Table 1). Thus, the NR+Mo activity, which is a indicator of the nutritional status of Mo (Shaked and Bar-Akiva 1967, Lavon and Goldschmidt 1999), increased significantly with 2 and 4 mM N supply (Table 1), with values being higher than those of initial NR and with a significant correlation between NR+Mo and Mo content ($r = -0.568$). There may be a hidden need for Mo in order to respond to higher N application to the plant. Nevertheless, the values for NR+NO₃⁻+Mo activity (Table 1), which is a bioindicator of the status of NO₃ and Mo (Lavon and Goldschmidt 1999), reflect a weak increase with 2 and 4 mM N supply, and an inverse correlation with the foliar Mo content ($r = -0.444$) was found. The results highlight

the strong influence of the environmental parameters (*i.e.* nitrogen supply) on the regulation of NR activity, influencing the nitrate reduction (*i.e.*, changes in the Mo and NO₃⁻ status) in leaves under lower N supply and thereby reflecting a possible increased NR synthesis for more efficient NO₃⁻ reduction to compensate for low N (Imsande and Touraine 1994). Therefore, we conclude that these different reactivated NR assays revealed a hidden deficiency of physiological imbalance of Mo in eggplants (Villora *et al.* 1998, Pulgar *et al.* 2000).

The application of 2 and 4 mM NH₄NO₃ as the N source, altered the Mo-NO₃⁻ relationship and distribution within the plant in comparison to 1 mM. These changes were offset by the altered NR activity in the leaf blades, with increased NO₃⁻ transport towards the fruits. In practical terms, the accumulation of NO₃⁻ in fruits (with the possibility of a subsequent reduction in fruit quality) must be avoided. However, despite the high NO₃⁻ content found in these plants, no loss of yield was detected (Villora *et al.* 1998). Nevertheless, excessive N applications must be excluded in a sustainable agriculture in the present and near future in order to improve the greenhouse production of eggplant. Biochemical indicators as NR proved to be valuable tools in the early nutritional diagnosis for greenhouse-grown plants.

References

- Andersen, P.C., Rhoads, F.M., Olson, S.M., Brodbeck, B.V.: Relationships of nitrogenous compounds in petiole sap of tomato to nitrogen fertilization and the value of these compounds as a predictor of yield. - *HortScience* **34**: 254-258, 1999.
- Cataldo, D.A., Haroon, M., Schrader, L.E., Young, V.L.: Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. - *Commun. Soil Sci. Plant Anal.* **6**: 71-80, 1975.
- Crawford, N.M.: Nitrate: nutrient and signal for plant growth. - *Plant Cell* **7**: 859-868, 1995.
- Chiallou, S., Morot-Gaudry, J.F., Salsac, L., Lesaint, C., Jolivet, E.: Compared effects of NO₃⁻ and NH₄⁺ on growth and metabolism on French bean. - *Physiol. vég.* **24**: 679-687, 1986.
- Gniazdowska, A., Krawczak, A., Mikulska, M., Rychter, A.M.: Low phosphate nutrition alters bean plants' ability to assimilate and translocate nitrate. - *J. Plant Nutr.* **22**: 551-563, 1999.
- Imsande, J., Touraine, B.: N demand and the regulation of nitrate uptake. - *Plant Physiol.* **105**: 3-7, 1994.
- Jaworski, E.J.: Nitrate reductase assay in intact plant tissues. - *Biochem. Biophys. Res. Commun.* **43**: 1274-1279, 1971.
- Jones, J.B., Jr.: Plant tissue analysis in micronutrients. - In: Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (ed.): *Micronutrients in Agriculture*. 2nd Ed. Pp. 477-521. SSSA, Madison 1991.
- Lavon, R., Goldschmidt, E.E.: Enzymatic methods for detection of mineral element deficiencies in citrus leaves: a mini-review. - *J. Plant Nutr.* **22**: 139-150, 1999.
- Marschner, H.: *Mineral Nutrition of Higher Plants*. 2nd Ed. - Academic Press, London 1995.
- Maynard, D.N., Barker, A.V., Minotti, P.L., Peck, N.H.: Nitrate accumulation in vegetables. - *Adv. Agron.* **28**: 71-118, 1976.
- Pulgar, G., Villora, G., Moreno, D.A., Romero, L.: Improving the mineral nutrition in grafted watermelon plants: nitrogen metabolism. - *Biol. Plant.* **43**: 607-609, 2000.
- Redingbaugh, M.G., Campbell, W.H.: Higher plant responses to environmental nitrate. - *Physiol. Plant.* **82**: 640-650, 1991.
- Sagi, M., Savidov, N.A., L'vov, N.P., Lips, S.H.: Nitrate reductase and molybdenum cofactor in annual ryegrass as affected by salinity and nitrogen sources. - *Physiol. Plant.* **99**: 546-553, 1997.
- Shaked, A., Bar-Akiva, A.: Nitrate reductase activity as an indication of molybdenum level and requirement of citrus plants. - *Phytochemistry* **6**: 347-350, 1967.
- Snell, F.D., Snell, C.T.: *Colorimetric Methods Analysis*. - Van Nostrand Company, New York 1949.
- Villora, G., Pulgar, G., Moreno, D.A., Romero, L.: Eggplant yield response to increasing rates of N-P-K fertilization. - *Phyton* **63**: 87-91, 1998.
- Wang, Z.Y., Tang, Y.L., Zhang, F.S.: Effect of molybdenum on growth and nitrate reductase activity of winter wheat seedling as influenced by temperature and nitrogen treatments. - *J. Plant Nutr.* **22**: 387-395, 1999.
- Zimmer, W., Mendel, R.: Molybdenum metabolism in plants. - *Plant Biol.* **1**: 160-168, 1999.