

BRIEF COMMUNICATION

Improving the mineral nutrition in grafted watermelon plants: nitrogen metabolism

G. PULGAR, G. VILLORA, D.A. MORENO and L. ROMERO

*Department of Plant Biology, Faculty of Sciences, University of Granada, E 18071 Granada, Spain***Abstract**

Watermelon (*Citrullus lanatus* [Trumb.] Mansfeld cv. Early Star), was used as scion grafted onto three cultivars of pumpkin (*Cucurbita pepo* L. cvs. Brava, Shintoza and Kamel) used as rootstocks and ungrafted Early Star plants were used as control. The rootstocks showed a high capacity for N uptake and transport to the scion where N reduction and assimilation improved growth of the scion in grafted plants with respect to the control.

Additional key words: amino acids, proteins, nitrate reductase, nitrite reductase.

The literature on the interaction between the rootstock and scion in grafted plants and its effect on mineral nutrition and yield is relatively scant (Villegas *et al.* 1992, Lee 1994). In relation to standard grafting techniques in *Cucurbitaceae*, the prevention of disease transmission (Kurata 1994) have been investigated, but few studies examine nutrition and related physiological changes in grafted plants (Agbaria *et al.* 1996,1998, Heo 1991). The overexploitation of resources under greenhouse cultivation at Almería (southern Spain) has resulted in the invasion of bacteria and fungi which deteriorate fruit quality and yield. One solution is the use of resistant species, such as the pumpkin, as a rootstock for other *Curcubitaceae* species (Ruiz *et al.* 1997). The aim of the present work was to ascertain the response of N metabolism of watermelon to grafting on different cultivars of pumpkin.

Pumpkin (*Cucurbita pepo* L.) cultivars Brava, Shintoza and Kamel, were used as rootstocks, and watermelon (*Citrullus lanatus* [Trumb.] Mansfeld) cv. Early Star, as the scion. The ungrafted cv. Early Star was used as control. Following Lee (1994), the grafts were made soon after germination of the rootstock seeds. Once the scar tissue had healed, the grafted plants were transplanted to plots in a greenhouse: 14-h photoperiod, irradiance of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, day/night temperature

25/18 °C, and relative humidity 60 - 80 %. The soil was loamy-sand (sand 37.3 %, silt 48.6 %, and clay 10.1 %), pH 8.24, electric conductivity 4.63 dS m^{-1} . The greenhouse was equipped with an interlinear drip fertigation system (the solution contained 0.24 M NH_4NO_3 , 0.18 M H_3PO_4 , 0.42 M KNO_3 , 0.07 M CaSO_4 , 0.03 M MgSO_4 , 10 μM Fe-EDDHA, 5 μM MnSO_4 , 2.5 μM ZnSO_4 , 1.25 μM CuSO_4 , and 0.5 μM MoSO_4 , 8 μM H_3BO_3 , pH 5.0 to 6.0). We used a randomized complete block design with 16 plots (18 m^2), each with 4 replications of 24 plants. We sampled only the 8 inner plants to avoid the edge effect. Samplings were carried out every 15 d (two mature leaves from the central part of the stem per plant). Leaves were washed with 1 % non-ionic detergent and rinsed three times with deionized water; 50 % of the sampled material was oven-dried at 70 °C for 24 h, ground and placed in plastic bags for further analysis, and 50 % of the fresh leaves was stored in a cold chamber at 4 °C. The NO_3^- and NH_4^+ were analysed in an aqueous extract (0.2 g d.m. in 20 cm^3 of Millipore water), shaking 120 min and finally centrifuging at 800 g for 5 min. The NO_3^- and NH_4^+ concentrations were measured using a UNICAM 8625 UV/VIS spectrometer (Unicam, Cambridge, UK) following Cataldo *et al.* (1975) and Baethgen and Alley (1989), respectively. Dry material (0.1 g) was digested in concentrated sulphuric

Received 15 February 2000, accepted 17 April 2000.

Abbreviations: EDTA- Na_2 - ethylenediaminetetraacetic acid disodium dihydrate; Fe-EDDHA - ferric ethylenediaminedi(o-hydroxyphenylacetic acid); NR - nitrate reductase; NiR - nitrite reductase.

Fax: (+34) 958 243254, e-mail: lromero@ugr.es

Table 1. Leaf concentration of NO_3^- , NH_4^+ and organic-N [$\text{mg g}^{-1}(\text{d.m.})$], and nitrate reductase (NR) [$\text{nmol}(\text{NO}_2^- \text{ formed}) \text{g}^{-1}(\text{f.m.}) \text{s}^{-1}$] and nitrite reductase (NiR) [$\text{nmol}(\text{NO}_2^- \text{ destroyed}) \text{g}^{-1}(\text{f.m.}) \text{s}^{-1}$] activities in watermelon plants. Means within the same column followed by different letter are significantly different at $P < 0.001$ ($n = 64$) by the Duncan's Multiple Range Test.

	NO_3^-	NH_4^+	Organic-N	NR	NiR
Control (Early Star)	11.41a	5.78a	26.40c	0.04b	9.59c
Brava \times Early Star	9.19b	1.09c	35.69a	0.20a	56.01a
Shintoza \times Early Star	7.78b	1.04c	33.33b	0.18a	55.30b
Kamel \times Early Star	8.24b	1.51b	31.03b	0.17a	55.11b
LSD _{0.05}	1.65	0.31	2.32	0.05	0.67

Table 2. Leaf content of soluble amino acids and proteins [$\text{mg g}^{-1}(\text{f.m.})$] and leaf fresh and dry masses [mg leaf^{-1}] in watermelon plants. Means within the same column followed by different letter are significantly different at $P < 0.001$ ($n = 64$).

	Soluble amino acids	Soluble proteins	Fresh mass	Dry mass
Control (Early Star)	0.09 d	2.20 c	5.19 c	0.83 b
Brava \times Early Star	0.54 a	15.51 a	7.46 a	1.14 a
Shintoza \times Early Star	0.37 b	15.34 a	6.53 b	1.09 a
Kamel \times Early Star	0.27 c	14.21 b	6.46 b	1.07 a
LSD _{0.05}	0.05	1.09	1.25	0.20

acid in the presence of H_2O_2 . After dilution with *Millipore* water, the organic-N was measured following Baethgen and Alley (1989) procedure. The enzymatic extract for determination of NR (EC: 1.6.6.1) and NiR (EC: 1.7.7.1) activities were obtained according to Becana *et al.* (1985). The fresh material was homogenized with 50 mM KH_2PO_4 buffer (pH 7.5), containing 2 mM EDTA- Na_2 , 2 mM dithiothreitol, 1.5 % (m/v) soluble casein and 1 % (m/v) insoluble polyvinylpyrrolidone. The homogenate was filtered and centrifuged at 3 000 g for 5 min and the supernatant centrifuged at 30 000 g for 20 min. For the determination of NR activity, we followed Kaiser and Lewis (1984) and Becana *et al.* (1985). The NO_2^- formed was determined colorimetrically at 540 nm according to Hageman and Hucklesby (1971). The NiR activity was determined by the disappearance of NO_2^- from the reaction medium (Lillo 1984). The resulting supernatant of samples in cold 50 mM KH_2PO_4 (pH 7) was centrifuged at 12 000 g for 15 min and used for the determination of soluble amino acids by the ninhydrin method (Yemm and Cocking 1955). Soluble proteins were measured following Bradford's method (Bradford 1976).

The leaf NO_3^- and NH_4^+ concentrations significantly differed between grafted and control plants, the highest concentration was registered by the control (Table 1). The leaf organic-N concentrations (Table 1) were greater in grafted plants than in control ones, the highest value being recorded for Brava \times Early Star (35 % higher than

the control), indicating that Brava was the rootstocks that really improved nitrogen uptake in the scion. Higher NR and NiR activities were found in leaves of grafted plants (Table 1), reflecting the greater efficiency of reducing NO_3^- to NO_2^- and NO_2^- to NH_4^+ , respectively. The NR stimulation in grafted plants has been ascribed also by Agbaria *et al.* (1996, 1998). The more efficient NR activity in grafted plants might be associated with the effect of rootstock on the uptake of water and nutrients (Heo 1991). The low leaf NO_3^- concentration in grafted plants may be due to higher NR, and thus a greater potential for reducing NO_3^- (Ruiz *et al.* 1997). The NiR activity was greater than NR, probably as a result of the toxic effect of the NO_2^- accumulation (Pécsvárdi and Szoldos 1996). The higher concentrations of NH_4^+ produced by higher NiR activities in the grafted plants favoured its rapid integration into amino acids and proteins (Barneix and Causin 1996). The highest concentrations of soluble amino acids and soluble proteins were recorded in the graft Brava \times Early Star (Table 2), supporting the enhanced capability for amino-acid and protein synthesis in the grafted plants. Moreover, the biomass production was higher in the grafts than in the control, reaching the highest fresh and dry masses in Brava \times Early Star graft (Table 2). This result confirms our hypothesis that the higher capability for N assimilation in grafted plants results in greater growth.

References

- Agbaria, H., Heuer, B., Zieslin, N.: Shoot-root interaction effects on nitrate reductase and glutamine synthetase activities in rose (*Rosa × hybrida* cvs. Ilseta and Mercedes) graftlings. - *J. Plant Physiol.* **149**: 559-563, 1996.
- Agbaria, H., Heuer, B., Zieslin, N.: Rootstock-imposed alterations in nitrate reductase and glutamine synthetase activities in leaves of rose plants. - *Biol. Plant.* **41**: 85-91, 1998.
- Baethgen, W.E., Alley, M.M.: A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant. - *Commun. Soil Sci. Plant Anal.* **20**: 961-969, 1989.
- Barneix, A.J., Causin, H.F.: The central role of amino acids on nitrogen utilization and plant growth. - *J. Plant Physiol.* **149**: 358-362, 1996.
- Becana, M., Aparicio-Tejo, P.M., Sánchez-Díaz, M.: Nitrate and nitrite reduction in the plant fraction of alfalfa root nodules. - *Physiol. Plant.* **65**: 185-188, 1985.
- Bradford, M.M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dyebinding. - *Anal. Biochem.* **72**: 248-254, 1976.
- 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.
- Hageman, R.H., Hucklesby, D.P.: Nitrate reductase. - *Methods Enzymol.* **23**: 497-503, 1971.
- Heo, Y.C.: [Effects of rootstocks on exudation and mineral elements contents in different parts of Oriental melon and cucumber.] - Thesis. Kyung Hee University, Kyung Hee 1991. [In Korean.]
- Kaiser, J.J., Lewis, O.A.H.: Nitrate reductase and glutamine synthetase activity in leaves and roots of nitrate fed *Helianthus annuus* L. - *Plant Soil* **70**: 127-130, 1984.
- Kurata, K.: Cultivation of grafted vegetables. II. Development of grafting robots in Japan. - *HortScience* **29**: 240-244, 1994.
- Lee, J.M.: Cultivation of grafted vegetables I. Current status, grafting methods and benefits. - *HortScience* **29**: 235-239, 1994.
- Lillo, C.: Diurnal variations of nitrite reductase, glutamine synthetase, glutamate synthetase, alanine aminotransferase and aspartate aminotransferase in barley leaves. - *Physiol. Plant.* **61**: 214-218, 1984.
- Pécsvárad, A., Zsoldos, F.: Nitrate reductase and nitrite reductase activity in nitrite- and chloride- rice seedlings. - *Plant Physiol. Biochem.* **34**: 659-663, 1996.
- Ruiz, J.M., Belakbir, A., López-Cantarero, I., Romero, L.: Leaf macronutrient content and yield in grafted melon plants: A model to evaluate the influence of rootstock genotype. - *Scientia Hort.* **71**: 227-234, 1997.
- Villegas, A., Mazuelos, C., Cantos, M., Troncoso, A.: [Influence of nitrogen on *in vitro* development of 161-49 grapevine rootstock.] - *Suelo Planta* **2**: 329-539, 1992. [In Span.]
- Yemm, E.W., Cocking, E.C.: The determination of amino acids with ninhydrin. - *Analyst* **80**: 209-213, 1955.