

## Effects of nutritive conditioning with n-forms on growth, proline and NRAsa activity in muskmelon seedlings

*Efectos del acondicionamiento nutritivo con formas nitrogenadas sobre el crecimiento, actividad de la prolina y NRAsa en plántulas de melón*

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### ABSTRACT

Galia melon seeds were primed with 8 g L<sup>-1</sup> of N as NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub>, or NH<sub>4</sub>NO<sub>3</sub>+KNO<sub>3</sub> or non-primed (Control). During germination, three solutions of differing water quality were used; one from "Almería" (SAR<sub>adj</sub> 2.0), and two to reproduce the water quality in "Pintados" in Atacama, northern Chile (SAR<sub>adj</sub> 10.4 and 14.3). Nutritive conditioning of seedling in the nursery was evaluated for increasingly saline waters. A base fertigation solution of 7.5 meq L<sup>-1</sup> NO<sub>3</sub><sup>-</sup>, 0.63 meq L<sup>-1</sup> H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 1.26 meq L<sup>-1</sup> SO<sub>4</sub><sup>=</sup>, 3.5 meq L<sup>-1</sup> K<sup>+</sup>, 4.14 meq L<sup>-1</sup> Ca<sup>2+</sup>, 1.26 meq L<sup>-1</sup> Mg<sup>2+</sup> and 0.5 meq L<sup>-1</sup> NH<sub>4</sub><sup>+</sup> was prepared with each water and alternated daily with a 7 mM solution of NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub> or NH<sub>4</sub>NO<sub>3</sub>+KNO<sub>3</sub> fertilizers. Seedlings were grown in perlite plugs in a passive greenhouse. Physical and chemical parameters, proline content and nitrate reductase activity were evaluated transplants and whose seed had been primed with the same N-forms. Seedlings fertigated with NH<sub>4</sub>NO<sub>3</sub> presented the highest plant size, number of leaves, leaf area, fresh and dry biomass, and the lowest water content both in leaf and whole transplant. Proline synthesis and nitrate reductase activity were not directly affected by water quality or by N-form conditioning. Proline synthesis decreased with seedling age, and the lowest values were obtained 34 days after sowing. Nevertheless, Nitrate Reductase activity was similar in all nursery stages.

**Keywords:** nutritive conditioning, seedlings, stress, enzymatic activity.

### RESUMEN

Las semillas de melón fueron primadas (acondicionamiento osmótico) con 8 g N L<sup>-1</sup> de N como NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub> y NH<sub>4</sub>NO<sub>3</sub>+KNO<sub>3</sub> y no primadas (Control). Durante la germinación se usaron tres soluciones con diferentes calidades de agua; una de "Almería" (RASadj 2,0) y dos que reproducen la calidad del agua en "Pintados" en Atacama, norte de Chile (RASadj 10,4 y 14,3). En semillero el acondicionamiento nutritivo de plántulas fue evaluado en aguas salinas. Una solución de fertirrigación base de 7,5 meq L<sup>-1</sup> NO<sub>3</sub><sup>-</sup>; 0,63 meq L<sup>-1</sup> H<sub>2</sub>PO<sub>4</sub><sup>-</sup>; 1,26 meq L<sup>-1</sup> SO<sub>4</sub><sup>=</sup>; 3,5 meq L<sup>-1</sup> K<sup>+</sup>; 4,14 meq L<sup>-1</sup> Ca<sup>2+</sup>; 1,26 meq L<sup>-1</sup> Mg<sup>2+</sup> y 0,5 meq L<sup>-1</sup> NH<sub>4</sub><sup>+</sup> fue preparado con cada agua y alternada diariamente con una solución de 7 Mm. con fertilizantes - NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub> y NH<sub>4</sub>NO<sub>3</sub>+KNO<sub>3</sub>. Las plántulas fueron cultivadas en bandejas con perlita en un invernadero pasivo. Parámetros físicos y químicos, contenido de Prolina y actividad de la Nitrato Reductasa fueron evaluados al transplante y cuya semilla había sido primada con las mismas formas nitrogenadas. Las plántulas fertirrigadas con NH<sub>4</sub>NO<sub>3</sub> presentaron el mayor tamaño de planta, número de hojas, superficie foliar, biomasa fresca y seca y el menor contenido de agua tanto en la hoja y en toda la planta. La síntesis de prolina y la actividad de la nitrato reductasa no fueron afectadas directamente por la calidad del agua y por el acondicionamiento con las formas nitrogenadas. La síntesis de Prolina disminuyó con la edad de la plántula, y los valores más bajos se obtuvieron a los 34 días después de la siembra. Sin embargo, la actividad de la Nitrato Reductasa fue similar en todas las etapas en el semillero.

**Palabras claves:** acondicionamiento nutritivo, plántulas, estrés, actividad enzimática.

### Introduction

Seedling production using water with high sodium and chloride content requires handling strategies that tend to lessen the negative effects and obtain seedlings with a high capacity to respond well to post-transplant stress.

Seed priming is one of the strategies used. However, results obtained in melon (Nascimento *et al.*, 1999), sorghum (Al-Mudaris *et al.*, 1999) and cucumber (Passam and Kakouriotis, 1994) indicate that seed priming treatments have no effect on later growth of the seedling, contrary to the results obtained in melon by Sivritepe *et al.*, (2003).

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Nursery Seedling production must fulfill two objective quality requirements: external appearance and post-transplant response. (Guzman, 2002).

With these aims in mind, the other strategy used is pre-transplant nutritional conditioning (PNC), which influences morphological and physiological seedling characteristics (Nicola and Basoccu, 1994). This nutritional conditioning helps to improve the vigor and fresh weight of the seedling, in which nitrogen plays an important role in post-transplant growth and early onset of production.

Nitrate Reductase (NR) is a key enzyme in all processes of nitrate assimilation by plants, controlling its mechanisms of synthesis and catalytic activity. Therefore, it is considered a limiting factor in the growth and development of plants (Kaiser *et al.*, 1999).

Jian and Hull (1998) and Fan *et al.* (2002) found that adding nitrate to the growth medium increased NR activity, more so in the leaves than in the roots.

Claussen (2002) found that in tomato plants, the form of nitrogen applied has a bearing on Proline content, which increases at greater concentrations in the nutritive solution and higher concentrations of  $\text{NH}_4^+$ . According to Heder (1999), if the total nitrogen concentration in the nutrient solution is kept constant, this amino acid acts as an indicator of osmotic stress.

## Materials and methods

This study was carried out on Galia Muskmelon seedlings grown in perlite plugs in a passive greenhouse. Seedlings came from seeds primed with  $8 \text{ g L}^{-1}$  of N as  $\text{NH}_4\text{NO}_3$ ,  $\text{KNO}_3$ , or  $\text{NH}_4\text{NO}_3+\text{KNO}_3$ . The control consisted of seedlings from non-primed seeds. All seeds were germinated in a rhizotron with three different solutions of water quality (Table 1), one from “Almería” ( $\text{SAR}_{\text{adj}}$  2.0), and two to reproduce the water quality in “Pintados”

in Atacama, northern Chile ( $\text{SAR}_{\text{adj}}$  10.4 and 14.3), and then sown in the nursery.

In the nursery, a base fertigation solution of  $7.5 \text{ meq L}^{-1} \text{ NO}_3^-$ ,  $0.63 \text{ meq L}^{-1} \text{ H}_2\text{PO}_4^-$ ,  $1.26 \text{ meq L}^{-1} \text{ SO}_4^{=}$ ,  $3.5 \text{ meq L}^{-1} \text{ K}^+$ ,  $4.14 \text{ meq L}^{-1} \text{ Ca}^{2+}$ ,  $1.26 \text{ meq L}^{-1} \text{ Mg}^{2+}$  and  $0.5 \text{ meq L}^{-1} \text{ NH}_4^+$  was prepared with each water type and alternated daily with a 7 mM solution of  $\text{NH}_4\text{NO}_3$ ,  $\text{KNO}_3$  or  $\text{NH}_4\text{NO}_3+\text{KNO}_3$  fertilizers, respectively.

Data were recorded on physical parameters (growth, tissue differentiation, biomass production, and water content) and biochemical parameters. Proline content was determined using the method of Bates *et al.* (1973), whereas nitrate reductase activity was measured by the method of (Jin *et al.* 2011).

All data were analyzed by the Statgraphics 5.1 © software using multifactorial ANOVA and LSD tests.

## Results and discussion

### Physical parameters of seedlings

The results presented correspond to the sampling 34 days after sowing and represent seedling characteristics and post-transplant resistance capacity.

Regarding the parameters of growth, tissue differentiation, fresh and dry biomass, and partitioning of dry biomass and water content, the main effect corresponds to the nutritional conditioning carried out with nitrogen forms (Tables 2 and 3), and there was no residual effect of priming treatments. This also coincides with the results obtained by Nascimento (2003).

For these parameters, the application of nitrogen forms partially or completely blocked the effect of the different water qualities used in nutritive solutions 2 and 3, both of which had a high sodium content ( $12.2$  and  $24.4 \text{ mmol L}^{-1}$ )

Table 1. Germination solution in ( $\text{meq L}^{-1}$ ).

Water quality	$\text{SO}_4^{=}$	$\text{Cl}^-$	$\text{K}^+$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$	$\text{Na}^+$	$\text{CE} - \text{dS m}^{-1}$
GS 1 <sup>1</sup> : Almería	0.81	1.5		1.2	1.0	1.3	0.5
GS 2: Pintados	6.16	18.12	0.68	12.10	3.34	12.20	2.6
GS 3: Pintados <sup>2</sup>	12.32	36.24	1.36	24.20	6.68	24.40	5.2

<sup>1</sup>GS: Germination solution; <sup>2</sup>Pintados: Double concentration.

Table 2. Variance analyses and LSD Test at 95% in the tissue growth and differentiation parameters in muskmelon Galia cv. Primal seedlings 34 days after sowing.

Variation factor	Parameters <sup>1</sup>				
	PH (cm)	SD (cm)	SI	Leaf Number	LA (cm <sup>2</sup> )
Fertigation	0.0124	0.0055	0.1309	0.0405	0.0118
-Control	4.43 a	0.42 a	10.57	3.11 a	56.11 a
-KNO <sub>3</sub>	5.12 bc	0.46 ab	11.26	3.67 ab	74.26 ab
-NK + NA <sup>2</sup>	4.89 ab	0.50 bc	9.77	3.33 ab	85.18 bc
-NH <sub>4</sub> NO <sub>3</sub>	5.62 c	0.53 c	10.68	3.89 b	101.76 c

<sup>1</sup> PH: Plant height; SDT: Stem diameter; SI: Slender Index; LA: Leaf area.

<sup>2</sup> NK + NA: KNO<sub>3</sub> + NH<sub>4</sub>NO<sub>3</sub>.

Means followed by different letters in the same column differ significantly (p < 0.05).

Table 3. Variance analyses and LSD test at 95 % in the Biomass Production and Water Content parameters in Muskmelon Galia cv. Primal seedlings 34 days after sowing.

Variation factor	Parameters <sup>1</sup>		
	Total Fresh Weight (g)	Total Dry Weight (g)	Total Water Content (%)
Fertigation	0.0044	0.0026	0.0038
-Control	5.29 a	0.35 a	93.4 c
-KNO <sub>3</sub>	7.25 ab	0.56 ab	92.5 bc
-NK + NA <sup>2</sup>	8.33 bc	0.75 bc	91.2 a
-NH <sub>4</sub> NO <sub>3</sub>	10.36 c	0.88 c	91.6 ab

<sup>1</sup> Including cotyledons; <sup>2</sup> NK + NA: KNO<sub>3</sub> + NH<sub>4</sub>NO<sub>3</sub>.

Means followed by different letters in the same column differ significantly (p < 0.05).

and chloride (18.12 and 36.24 mmol L<sup>-1</sup>). Only in nutritive solution 1, which had lower EC, did plants show significantly greater height.

This blocking effect is positive, especially with the application of the different nitrogen forms, and notably with NH<sub>4</sub>NO<sub>3</sub>. In this case, seedlings presented the best quality characteristics for a post-transplant response, showing greater vigor (Preciado *et al.*, 2002), due to their greater height, stem diameter, leaf number, leaf area, although the shoot/root ratio showed no differences between fertigation treatments (Table 4).

This greater vigor in seedlings conditioned with NH<sub>4</sub>NO<sub>3</sub> is probably due to the synergy between the NO<sub>3</sub><sup>-</sup> anion and the NH<sub>4</sub><sup>+</sup> cation, as recorded by Kronzucker *et al.* (1999). The greater stem diameter and leaf area of these seedlings have a direct incidence on the higher biomass percentage in the shoot, which constitutes the main temporal sink. These characteristics are reported by Preciado *et al.* (2002) and Cuartero

Table 4. Variance analyses and LSD test at 95 % in the Shoot/Root ratio parameter in Muskmelon Galia cv. Primal seedlings 34 days after sowing.

Variation factor	Shoot/Root Ratio
Fertigation	0.5053
-Control	4.61
-KNO <sub>3</sub>	4.66
-NK + NA <sup>2</sup>	4.40
-NH <sub>4</sub> NO <sub>3</sub>	3.85
Nutrient Solution (SAR) <sup>1</sup>	0.0135
-ND1: 2	3.59 a
-ND2: 10	4.30 ab
-NT3: 14	5.22 b

<sup>1</sup> Prepared based on three different levels of water quality.

Means followed by different letters in the same column differ significantly (p < 0.05).

and Fernández-Muñoz (2002), as being relevant for seedling vigor and for higher tolerance to post-transplant stress. The results obtained indicate

that the nitrogen supplement applied improves seedlings' physiological response.

Moreover, seedlings from the nutritional treatment with  $\text{NH}_4\text{NO}_3$  show the greatest total fresh and dry weight, as well as the greatest weight root, stem, and leaf (Table 3). Using this treatment, the highest percentages of dry biomass in the root and stem, which is particularly important as a reserve of nutrients and for better tolerance to post-transplant stress.

The higher level of EC in the nutritive solutions only had a significant effect on the plant's height. The other parameters of growth and tissue differentiation were not affected; although the overall size of the plant was less with a nutritive solution 3; this treatment showed the highest shoot/root ratio: 5.2, which is 1.4 times greater than with nutritive solution 1 (Table 4). This result shows that this indicator of seedling vigor could also be used as an indicator of stress.

### Biochemical parameters

The fertigation solutions affected Proline synthesis, but there was no direct relationship with the increase in  $\text{SAR}_{\text{adj}}$ ; on the other hand, the Nitrate Reductase activity was not affected.

Stress levels of the melon seedlings 34 days after sowing were lower, with less Proline synthesis (Figure 1). The lower Proline synthesis content obtained with nutritive solution 3 is not related to this solution's higher EC, which indicates that the supplementary fertigation has minimized the osmotic stress level. However, when conditioning is carried out with sodium chloride, there is a positive correlation between the concentration applied and Proline content (Sivritepe *et al.*, 2003).

Nitrate Reductase Activity, both endogenous and induced, presented erratic behavior, showing

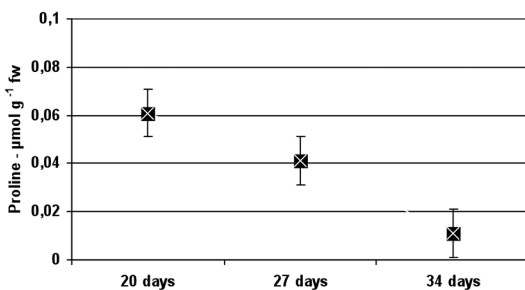


Figure 1. Evolution of Proline content in Muskmelon Galia cv. Primal seedlings.

higher endogenous activity in the control group than in the nitrogen enhanced treatments and greater induced activity in nutritive solutions 1 and 2 (Table 5). Although no significant differences are obtained in the relationship between induced and endogenous activity, there is a tendency to obtain the highest relationship with the  $\text{NH}_4\text{NO}_3$  fertigation treatment and nutritive solution 1.

Irrespective of the apportion and source of nutrients, endogenous Nitrate Reductase (ENR) activity was the same on the three sampling dates. However, the activity of the induced form of the enzyme (INR) showed an increase after 27 days due to reserves of non-metabolized  $\text{NO}_3^-$ , which fall 4.5-fold on the last sampling date (Figure 2). The INR/ENR ratio (Figure 3) follows the trend of the INR, with values in the normal range over all three samplings. These results are in line with those of Valenzuela *et al.* (1991).

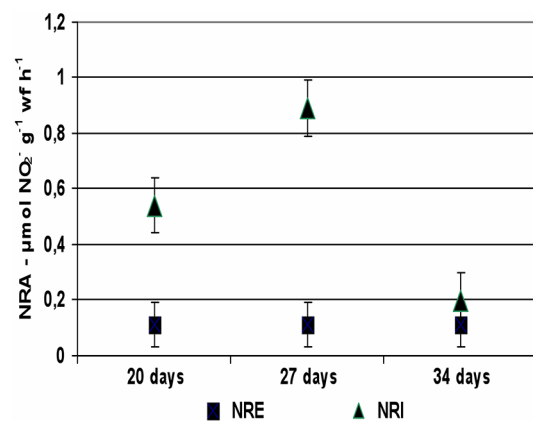


Figure 2. Evolution of Nitrate Reductase Activity in Muskmelon Galia cv. Primal seedlings.

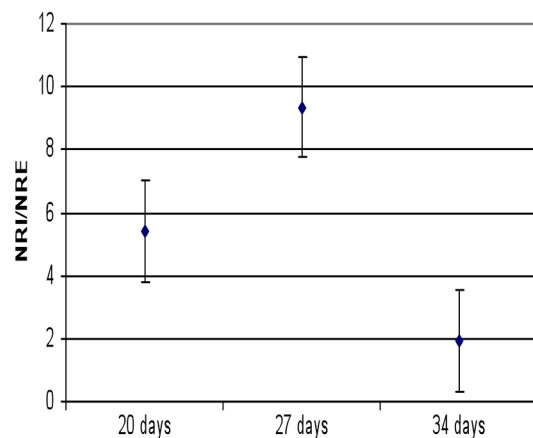


Figure 3. INR/ENR ratio in Muskmelon Galia cv. Primal seedlings.

Table 5. Variance Analyses and LSD Test at 95% in the Nitrate Reductase Activity and Proline production in Muskmelon Galia cv. Primal seedlings 34 days after sowing.

Variation Factor	Parameters			
	ENR <sup>2</sup> ( $\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ wf h}^{-1}$ )	INR <sup>3</sup> ( $\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ wf h}^{-1}$ )	NRI/NRE	Proline ( $\mu\text{mol g}^{-1} \text{ wf}$ )
Nutrient Solution (SAR) <sup>1</sup>	0.2166	0.0138	0.1390	0.0014
- ND1: 2	0.10	0.21 b	2.10	0.018 b
- ND2: 10	0.12	0.23 b	2.03	0.015 b
- NT3: 14	0.09	0.15 a	1.67	0.008 a

<sup>1</sup> Prepared based on three different levels of water quality.

<sup>2</sup> ENR: Endogenous Nitrate Reductase.

<sup>3</sup> INR: Induced Nitrate Reductase.

Means followed by different letters in the same column differ significantly ( $p < 0.05$ ).

Nitrate Reductase activity is 3 to 5 times less than the values found by Ruiz and Romero (1999) in grafted melon grown in non-saline medium (EC: 2.03 dS m<sup>-1</sup>), and 10 ten times less than those obtained by Valenzuela *et al.* (1991). The low values obtained are due to synthesis inhibition of the enzyme as a result of the EC of the fertigation solutions (EC: 3 to 6 dS m<sup>-1</sup>). This synthesis inhibition was also reported by Dubey and Pessaraki (2001), who pointed out a drop in the reduction of NO<sub>3</sub><sup>-</sup> due to less availability since the excess is stored in the vacuole (Ulrich, 2002).

## Conclusions

Seedlings fertigated with NH<sub>4</sub>NO<sub>3</sub> presented the highest plant size, number of leaves, leaf area, fresh, and dry biomass and the lowest water content both in leaf and whole transplant. Proline synthesis and nitrate reductase activity were not directly affected by water quality or by N-form conditioning. Proline synthesis decreased with seedling age, and the lowest values were obtained 34 days after sowing. Nevertheless, Nitrate Reductase activity was similar in all nursery stages.

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