Net Journal of Agricultural Science
Vol. 6(4), pp. 63-69, November 2018
DOI: 10.30918/NJAS.64.18.031
ISSN: 2315-9766
Full Length Research Paper

Effects of the ionic imbalance in the fertigation solution on growth, proline content and nitrate reductase activity in muskmelon seedlings

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Accepted 26 November, 2018

ABSTRACT

The aim of this research was to evaluate the effects of ionic imbalance of fertigation solution in muskmelon (*Cucumis melo*) seedlings on growth, proline content and nitrate reductase activity (NRA). 'Galia' melon seeds were primed with 8 g·L^{-1} of N different N-form fertilizers (NH_4NO_3, KNO_3 or NH_4NO_3+KNO_3), or non-primed (Control). These seeds were germinated and grown in perlite inside a passive greenhouse nursery. The water quality of "place Pintados region Tarapacá - Chile" was reproduced (EC: 2.6 dS m^{-1}, pH: 7.3; Na^+: 12.2 meq L^{-1}, Cl^-: 18.12 meq L-1, SAR_{adj}: 10.4). Four fertigation solutions were prepared with this water, maintaining a $K^+/(Ca^{2+} + Mg^{2+})$ ratio of 0.4 and pH between 6.1 and 6.6, but modifying EC (3.0; 4.1; 5.1; 6.2), SAR_{adj} (10.4; 9.2; 8.1; 7.0) and NO_3^-/Cl^- ratio (0.47;0.73;0.86;1.25). The ionic ratio in fertigation solutions was modified during germination and seedling growth, which led to an increase of NO_3^- from 8.6 to 22.8 meq L^{-1}. Seedlings fertigated with 5.1 dS m^{-1} (SAR_{adj}: 8.1) nutrient solution showed the highest plant height, leaf number and leaf area, but there was no significant effect on fresh and dry biomass. Proline content and NRA showed a high correlation with NO_3^- concentration. The lowest proline content was obtained at 12 meq L^{-1} NO_3^- in the fertigation solution, indicating the lowest stress level and the highest efficiency in nitrogen absorption. Moreover, proline levels decreased before seedlings were planted. This different behaviour between endogenous and induced NRA may be useful to optimize NO_3^- supply and management in the nursery.

Keywords: Nutrient solution, salt stress, proline content, nitrate reductase activity.

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INTRODUCTION

The saline conditions of water and soil in the Atacama Desert of northern Chile affect crop establishment and yield, such as muskmelon Galia, that cause physiological damage by excessive accumulation of sodium and chloride in vegetative organs (Mirck and Zalesny, 2015; López et al., 2017).

Calcium is fundamental to physiological processes and cation/anion balance at different cell-level. Potassium to enhance nitrogen absorption and metabolism (Helal et al., 1975) and is also fundamental to maintain the osmotic potential (Akinci and Simsek, 2004), and these authors obtained beneficial effects on root and vegetative growth for cucumber plants cv. Beith Alpha F1 in vitro and grown in NaCl salinized solutions supplemented with K+ and Ca2+ applications. These results were similar to those obtained by Navarro et al. (1999) for melon and Kaya et al. (2002), for strawberry.

Shirazi et al. (2005) also obtained increased root and vegetative growth in different wheat cultivars maintaining a high K^+/Na^+ ratio, attributing this positive response to the effects of competition between K^+ and Na^+ at the absorption sites located in the root cell plasmalema.

This modification of the ionic balance implies a greater supply of cations and anions which increase the osmotic potential of the fertigation solutions, thereby modifying nitrate reductase activity (NRA) and proline synthesis (Clauseen, 2002).

Regarding to Proline, numerous studies have shown that this amino acid increases in higher plants under different environmental stresses, generating osmoregulatory processes and osmotic adjustment (Heder, 1999), osmoprotectants (Roosens et al., 1999), including salinity (Szabados and Savoure, 2009). Greater Proline synthesis has been reported in tomato (Clauseen, 2002) and in melon (Sivritepe et al., 2003) with greater supply of N-NH_4^+ and NaCl, respectively in fertigation solutions. Also, in tomato, Flores et al. (2000) reported that absorption of NO_3^- and NRA diminished due to a specific effect of the salts rather than the osmotic potential.

The present study proposes a strategy of modifying the ionic balance in fertigation solutions in order to minimize the antagonistic effects among ions and their negative influence on post-transplant muskmelon seedling quality on growth, and content proline and NRA. Cations and anions are added to reduce the effect of salinity due to NaCl in the nutritive solution.

MATERIALS AND METHODS

Priming seed pre-treatments

Muskmelon (*Cucumis melo*) seeds (Galia type cv. Primal) were primed for four days at 20°C with 8 g L^{-1} of different N-form fertilizers: NH_4NO_3 (AN); KNO_3(KN) or NH_4NO_3+KNO_3 (AN+KN) or non-primed (Control) as described by Sivritepe et al. (1999). After priming, all seeds were washed with tap water and rinsed with distilled water. Then they were dried between two filter papers and set to germinate in rhizotrons in the laboratory. Blocks of 30 germinated seeds from each priming treatment were sowed in polystyrene cell plug trays, using perlite substrate, covered with a thin layer of vermiculite to maintain the humidity. Plants were grown in a passive greenhouse nursery.

SAR treatments

Primed and control seeds were germinated in four fertigation solutions (FS) with different level of SAR_{adj} (Table 1), prepared from the saline water of Pintados (north Chile). The chemical composition of Pintados saline water was: 2.59 meq L^{-1} HCO_3^-; 6.1meq L^{-1} SO_4^-; 18.1 meq $L^{-1}$$Cl^-$; 0.7meq L^{-1} K^+; 12.1 meq L^{-1} Ca^{2+}; 3.3meq L^{-1} Mg^{2+}; 12.2 meq L^{-1} Na^+; SAR_{adj}: 10.4 and EC: 2.6 dS m^{-1}. These four treatments of FS were obtained maintaining a constant relation $K^+/(Ca^{2+}+Mg^{2+})$=0.4,0.5 meq $l^{-1}$of HCO_3^- and pH (between 6.1 and 6.6), but modifying SAR_{adj} (10.4; 9.2; 8.1; 7.0), EC, osmotic potential (Table 1) and ionic ratios (Table 2).

Each FS treatment was applied to the seedlings daily for 36 days with a micro sprinkler of 50 l h^{-1}. Volume and frequency of fertigation was adjusted according to the greenhouse environmental conditions and to the seedlings growth. The nursery fertigation management was divided into three stages taking as base the control treatment. The first stage was up to the first true leaf, at which 1/3 concentration of each FS was applied. The second stage started with the second true leaf at 2/3 of the FS concentration and the third stage started with the third true leaf, when the final concentration of each was applied (Table 1).

Analysis and determinations

Four destructive samplings were carried out, one each week. Plant growth and biochemical measurements were performed. Measurements of seedling, 36 days after sowing, were plant height (PH), stem diameter (SD), leaf number (Leaf Nº), leaf area (LA); fresh weight of root, stem and leaves (FRW, FSW, FLW, respectively); and dry weight of root, stem and leaves (DRW, DSW, DLW). Leaf area was determined from digitalized images of leaves using the Idrisi © computer program.

The following quality indices were determined: slenderness index (SI) (plant height/diameter) (Sánchez-Gómez et al., 2006); root/shoot ratio (dry weight basis), total fresh weight (TFW = FRW + FSW + FLW), total dry weight (TDW = DRW + DSW + DLW), percentage of water content (%WC = 100 - (TDW/TFW*100), specific leaf area (SLA = LA/DLW)and root density (root dry weight). Nutrient assimilation efficiency was assessed with an index defined as the average nutrient content (root + shoot)/total fertilizer amount applied throughout the growing cycle.

The biochemical measurements carried out to evaluate stress and the enzymatic activity levels were proline content, according to the method of Bates et al. (1973) and Nitrate Reductase Activity (NRA). Endogenous (ENR) and NO_3^-induced (INR) were carried out according to Bar Akiva, and adapted by Valenzuela (Sanchez, 1993).

Statistical analyses

Data analysis was carried out using Statgraphics Centurion XVI 16.1.17 © package. A factorial randomised plots design was used. Two factors (Priming and Fertigation Solution SAR_{adj}) were considered. The values of %WC content were arcs in-transformed and Leaf Nºlog was transformed using $log_{10}(X+1)$. After ANOVA testing, means with significance differences p<0.05, were separated by the LSD test (&=0.05).

Table 1. Fertigation solutions (FS) obtained using "Pintados" saline water (meq L^{-1}).

SAR_{adj}	$H_2PO_4^-$	NO_3^-	$SO_4^=$	Cl^-	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	Na^+	EC*	OP**	pH
FS1=10.4	2.0	8.6	6.1	18.1	6.8	12.1	3.3	0.9	12.2	3	-0.15	6.6
FS2=9.2	2.0	13.3	6.1	18.1	8.3	12.1	6.6	1.2	12.2	4	-0.17	6.6
FS3=8.1	4.6	15.6	14.0	18.1	12.0	18.0	9.0	1.6	12.2	5	-0.23	6.3
FS4=7.0	6.8	22.8	18.3	18.1	16.0	24.0	12.0	2.3	12.2	6	-0.29	6.1

*EC= electric conductivity (dS m^{-1}): **Osmotic Potential (MPa) according to Rawlins (1981).

Table 2. Ionic ratios of the fertigation solutions (FS).

SAR_{adj}	Na^+/K^+	Na^+/Ca^{2+}	Na^+/Mg^{2+}	NO_3^-/Cl^-
FS1=10.4	1.8	1.0	3.7	0.5
FS2=9.2	1.5	1.0	1.8	0.7
FS3=8.1	1.0	0.7	1.4	0.9
FS4=7.0	0.8	0.5	1.0	1.3

RESULTS

All data presented correspond to seedling sampling 36 days after sowing. The response obtained in seedlings was due to the combined effect of fertigation solutions (NO_3^-, K^+, SAR_{adj}, and EC) and osmotic priming (N-form). Previous priming had residual effects modifying seedling response.

Physical parameters of seedlings

The greatest growth, tissue differentiation (PH, TI, and LA) were obtained with FS3 treatment (SAR_{adj} = 8.1; EC = 5 dS m^{-1}; NO_3^- = 15.6 meq L^{-1} and K^+ = 12.0 meq L^{-1}) and on AN primed seeds (Table 3).

No significant effects on biomass production (TFW and TDW) due to fertigation solution were observed, whereas water content was higher for FS2 and FS3 treatment (Table 4). The residual effect of priming are evident from the highest values obtained for TFW, TDW and WC%on ammonium nitrate (AN) primed seeds.

Seedlings from seeds primed with AN showed (Table 5) the highest shot/root ratio (7.53) and SLA (193.59) and lowest root/stem ratio (0.59). Melon seedlings fertigated with FS3 (12 meq L^{-1} of K^+ and 15.63 meq L^{-1} of NO_3^-) also showed the highest shoot/root ratio (8.09), SLA (200) and the lowest root/stem ratio (0.52).

Table 3. ANOVA and LSD Test at 95% for tissue growth and differentiation parameters for primed muskmelon seed, irrigated with different fertigation solution (FS) for 36 days after sowing.

Factor	Parameters[1]				
	Plant height (cm)	Stem diameter (cm)	SI	Leaf Nº	LA (cm^2)
Priming	0.0005*	0.1637ns	0.0084*	0.1390ns	0.0135*
Control	10.65^b	0.40	26.44^{ab}	4.88	119.64^b
KN (KNO_3)	11.08^b	0.33	33.19^b	4.88	90.33^{ab}
AN (NH_4NO_3)	12.84^b	0.41	33.59^b	5.13	116.65^b
KN+AN	5.98^a	0.30	20.85^a	4.38	65.00^a
Fertigation solution (SAR_{adj})	0.0004*	0.1748ns	0.0016*	0.0750ns	0.1765ns
FS1=10.4	10.21^b	0.34	30.23^a	4.88	106.10
FS2=9.2	9.30^{ab}	0.43	22.77^a	4.75	86.55
FS3=8.1	14.16^c	0.37	38.2^{7b}	5.25	116.05
FS4=7.0	6.86^a	0.30	22.81^a	4.38	82.92
Priming * SAR_{adj}	0.0336*	0.3884ns	0.0695ns	0.0827ns	0.1914ns

[1] SI = Slenderness Index; LA = Leaf Area.

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

Biochemical parameters

Proline content and NRA showed a differential response to the modification of SAR_{adj} and the application of NO_3^- in the FS treatment.

Proline content was not affected by the residual effect of priming. Highest values were obtained with the highest NO_3^- application in FS4 (22.8 meq L^{-1}) corresponding to lowest SAR_{adj} (7.0) and EC (6 dS m^{-1}) (Table 6). The lowest values of Proline content were found at 13.3 meq L^{-1} of NO_3^- applied in the FS2 treatment and the fitted function (R^2 = 0.999) shows (Figure 1) an inflection point calculated at 12 meq L^{-1} of NO_3^- (Table 6). Proline content increased when both a lower and higher concentration of $NO_3^-$was applied above these values. Proline content also showed (Figure 2) a high correlation with EC of FS, fitting a quadratic function (R^2 = 0.959) with a minimum at 4 dS m^{-1}.

Table 4. ANOVA and LSD Test at 95% for biomass production and water content parameters, for primed muskmelon seed, irrigated with different fertigation solution (FS) for 36 days after sowing.

Variation factor	**Parameters**		
	Total fresh weight (g)	**Total dry weight (g)**	**Total water content (%)**
Priming	0.0129*	0.0487*	0.0081*
Control	11.87^{b}	1.12^{b}	90.47^{a}
KN (KNO_3)	9.39^{ab}	0.93^{ab}	90.04^{a}
AN (NH_4NO_3)	12.02^{b}	1.00^{b}	91.80^{b}
KN+AN	7.12^{a}	0.67^{a}	90.35^{a}
Fertigation solution (SAR_{adj})	0.4363ns	0.3236ns	0.0014*
FS1=10.4	10.67	1.07	89.50^{a}
FS2=9.2	9.37	0.80	91.49^{b}
FS3=8.1	11.25	0.97	91.39^{b}
FS4=7.0	9.12	0.88	90.29^{a}
Priming * SAR_{adj}	0.2751ns	0.4284ns	0.0837ns

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

Table 5. ANOVA and LSD Test at 95% for parameter ratios for primed muskmelon seed, irrigated with different fertigation solution (FS) for 36 days after sowing.

	Parameter ratios		
	Shoot/root	**Root/Stem**	**SLA^{1}**
Priming	0.03*	0.01*	0.01*
Control	6.66^{ab}	0.68^{a}	176.58^{ab}
KN (KNO_3)	6.63^{ab}	0.64^{a}	169.09^{a}
AN (NH_4NO_3)	7.53^{b}	0.59^{a}	193.59^{b}
KN+AN	5.42^{a}	0.87^{b}	166.59^{a}
Nutrient Solution (SAR)	0.01*	0.01*	0.00*
FS1=10.4	6.30^{a}	0.68^{a}	152.94^{a}
FS2=9.2	5.73^{a}	0.78^{b}	186.85^{b}
FS3=8.1	8.09^{b}	0.52^{a}	200.15^{b}
FS4=7.0	6.12^{a}	0.78^{b}	155.90^{a}
Priming * SAR_{adj}	0.32ns	0.12ns	0.11ns

[1] SLA = Specific Leaf Area = (LA/DLW)

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

Nitrate reductase activity (both endogenous and induced) showed (Figure 3) the minimum activities of their residual effect on AN primed seeds. Increases in NRA (both ENR and INR) were observed, with SAR values decreasing in each of the tested FS.

These results show that seedlings that could easily withstand post-transplant stress were those of FS3 treatment (SAR_{adj} 8.1, 15 meq L^{-1} of NO_3^- and EC 5 dS m^{-1}), because they have greatest growth and development parameters, and low Proline levels. On the other hand, considering that the NRA is more related to the content of NO_3^- and ENR/INR ratio can be interpreted as an indicator of the Nitrate Use Efficiency, optimal NO_3^- efficiency was obtained in 15.6 meq L^{-1} of NO^{3}- FS applications.

DISCUSSION

These results (Table 3) differ from those obtained by Sivritepe et al. (2003), Franco et al. (1993) and Franco et al. (1997), who attribute the reduction in growth and

Table 6. ANOVA and LSD Test at 95% for nitrate reductase activity and proline content for primed muskmelon seed, irrigated with different fertigation solution (FS) for 36 days after sowing.

Variation factor	Parameters			
	ENR[1] (µmol NO_2^- g^{-1} wf h^{-1})	**INR[2] (µmol NO_2^- g^{-1} wf h^{-1})**	**INR/ENR**	**Proline (µmol g^{-1}wf)**
Priming	<0.00*	<0.00*	<0.00*	0.13ns
Control	0.36^d	0.47^c	1.43^b	0.05
KN (KNO_3)	0.24^b	0.43^b	2.09^d	0.09
AN (NH_4NO_3)	0.21^a	0.36^a	1.71^c	0.07
KN+AN	0.25^c	0.35^a	1.19^a	0.06
Fertigation Solution (SAR_{adj})	<0.00*	<0.00*	<0.00*	<0.00*
FS1=10.4	0.12^a	0.21^a	1.91^c	0.06^a
FS2=9.2	0.20^b	0.33^b	1.60^b	0.04^a
FS3=8.1	0.27^c	0.34^b	1.22^a	0.04^a
FS4=7.0	0.47^d	0.72^c	1.69^b	0.13^b
Priming * SAR_{adj}	<0.0001*	<0.0001	<0.0001	0.0784ns

[1]ENR: Endogenous Nitrate Reductase activity;[2]INR: Induced Nitrate Reductase activity.
Means followed by different letters in the same column differ significantly (p<0.05).

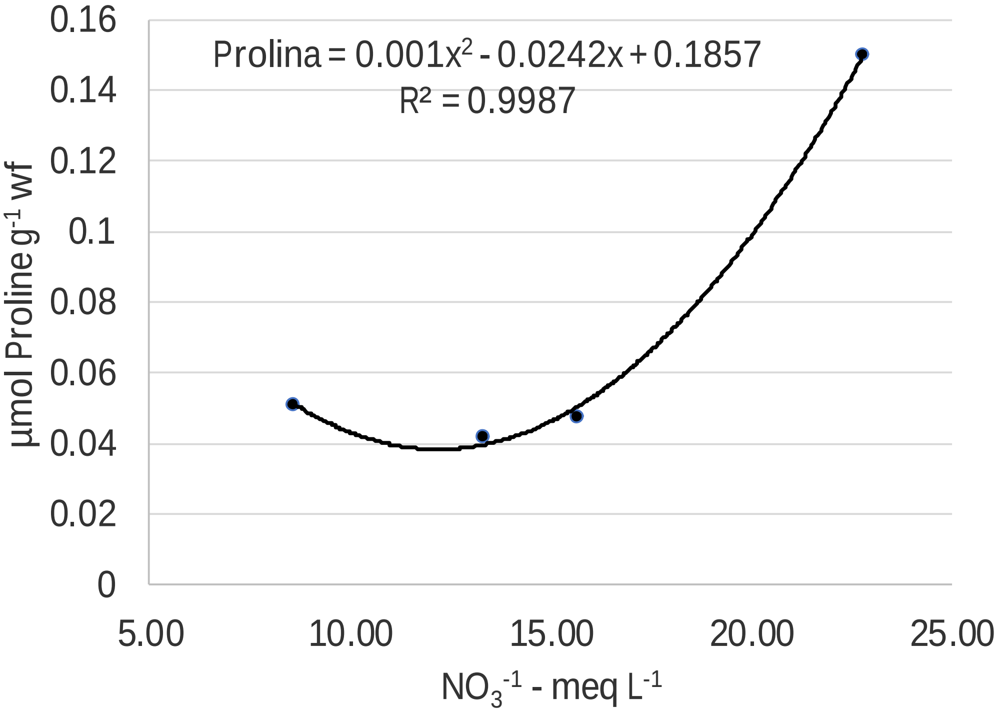

Figure 1. Proline response in function of nitrate supply in the fertigation solution.

development of melon seedlings to the increase in EC due to NaCl. On the other hand, Preciado et al. (2002) attribute the observed reduction to the high supply of NO_3^-, K^+ and elevated EC. The negative effects of EC are compensated with adequate SAR and NO_3^-/ K^+ ratios.

The results obtained in biomass production (Table 4) on melon seedlings differ from those previous (Franco et al., 1993; Franco et al., 1997; Sivritepe et al., 2003) which attribute lower biomass production to the increase in EC due to NaCl. They also differ from those of Wahome (2003), who points out that greater salinity affects biomass production in crops as a whole. The results evidence a compensatory effect between the residual effect of priming and the modification of the ionic

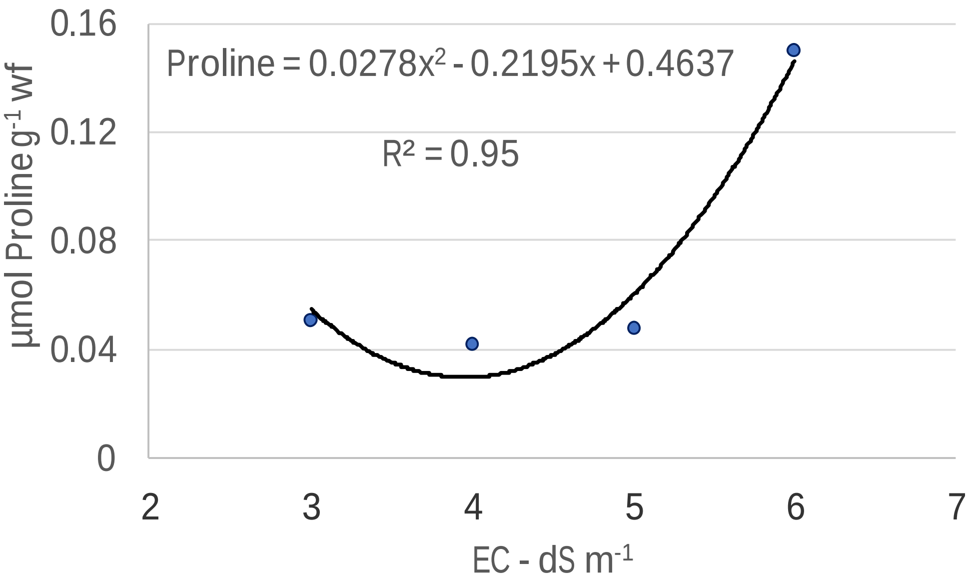

Figure 2. Proline response in function of electrical conductivity(EC) on the fertigation solution.

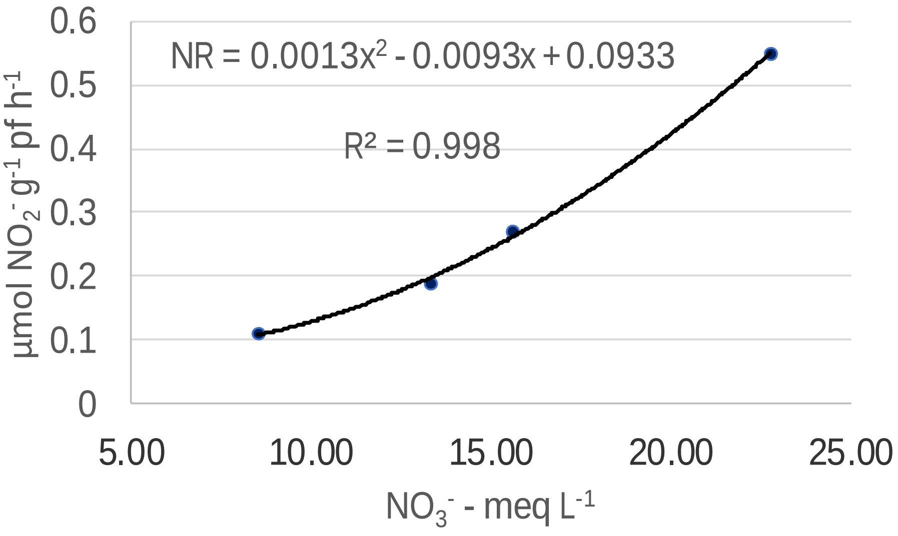

Figure 3. Nitrate Reductase Activity in function of nitrate supply in the fertigation solution.

ratios of FS, blocking the action the EC, increased by NaCl.

Our results about melon seedlings vigour (Table 5), are in agreement with those of Preciado et al. (2002), indicating greater plant vigour, which are positively correlated with a higher post-transplant stress resistance (Filgueira et al., 1998; Cuartero and Fernández Muñoz, 1999).

Proline production coincides with that obtained by Sivritepe et al. (2003) in melon seedlings primed with NaCl, who found an inflection point for proline at 4.5 dS m^{-1} EC. Seed tolerance to sodium chloride post priming germination is linked to the concept of priming memory associated with the action of specific genes and enzymes that modulate proline synthesis (Kubala et al, 2015; Martinez et al., 2016)

NR activity values are lower than those obtained by Valenzuela et al. (1991), for melon plants at harvest. The INR/ENR ratio showed the lowest values at FS3 (SAR_{adj}=8.1), indicating highest NO_3^- reduction efficiencies. NRA content (Figure 3) also fitted a highly correlated quadratic function (R^2 = 0.999) with NO_3^- supply in FS. These results coincide with the lineal function in a range from 0 to 5 mM of NO_3^- obtained in previous studies (Jiang and Hull, 1998; Ouko, 2003) but indicate that there was a basal activity of NRA without nitrogen supply.

In conclusion, the seedlings fertigated with 5 dS m^{-1} (RAS_{adj}: 8.1) in FS showed the highest plant height, leaf number and leaf area, but biomass production was not significantly affected. Proline content and NRA fit a high correlation with NO_3^- supply, determining the lowest stress and the highest efficiency level, respectively. The lowest proline content was calculated at 12 meq L^{-1} NO_3^- in FS. The ratio between endogenous (ENR) and induced (INR) NRA may be useful to optimize temporary management of NO_3^- supply in nursery.

ACKNOWLEDGEMENTS

Thanks to SYNGENTA Seeds S.A. for supplying the muskmelon Primal (MG591) F1 hybrid seeds and Centro de Investigación y Desarrollo en RecursosHídricos (CIDERH) R09I1001.

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Citation: Olave J, Guzman M, Sanchez A, Santander Ch., 2018. Effects of the ionic imbalance in the fertigation solution on growth, proline content and nitrate reductase activity in muskmelon seedlings. Net J Agric Sci, 6(4): 63-69.