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Original Article

Effect of the concentration and ionic form of nitrogen (N) on photosynthesis, growth and fruit production of blueberry (*Vaccinium corymbosum* L.)

Efecto de la concentración y forma iónica del nitrógeno (N) en la fotosíntesis, crecimiento y producción de frutos de arándano (*Vaccinium corymbosum* L.)

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ABSTRACT

The aim of this work was to evaluate the main effects and the interactions of nitrogen (N) concentration and ammonium (NH_4^+): nitrate (NO_3^-) proportion in the nutrient solution, on net photosynthesis, plant growth, production and quality of fruits on blueberry (*Vaccinium corimbosum* L.) plants. The studied factors were N concentration (0.6 and 6.0 mM) and NH_4^+: NO_3^- proportion (100 % NH_4^+, 50 % NH_4^+ - 50 % NO_3^- and 100 % NO_3^-). 243 d after the experiment was established, net photosynthesis (PN) was measured and a day after, leaf area (LA), shoots fresh weight (SFW) and roots fresh weight (RFW) were determined. Fruit yield (FY), fruit diameter (FD) and Brix degrees (°Brix) were evaluated in four harvests along fruit production period. The results showed higher values on PN, LA, SFW, FY and FD in plants that received 6.0 mM N as NH_4^+; nevertheless, N concentration altered the NH_4^+: NO_3^- proportion effects and at 0.6 mM N only FY and FD maintained such a pattern. It is concluded that N concentration modifies the effect of NH_4^+: NO_3^- proportion and it is proposed that the effects of both factors on photosynthesis, growth and fruit production of blueberry were mediated by their interaction with plant carbohydrates availability.

Keywords: nitrate; ammonium; quality; yield; carbohydrates.

RESUMEN

En este trabajo se evaluó la concentración de nitrógeno (N) (0.6 y 6.0 mM), la proporción de amonio (NH_4^+): nitrato (NO_3^-) (100 % NH_4^+, 50 % NH_4^+ - 50 % NO_3^- y 100 % NO_3^-) y su interacción, sobre la fotosíntesis neta, el crecimiento de las plantas, la producción y la calidad de los frutos, en arándano (*Vaccinium corimbosum* L.). Las plantas se mantuvieron por 243 d en un sistema hidropónico y después se midió la fotosíntesis neta (FN), el área foliar (AF), el peso fresco de los brotes (PFB) y el peso fresco de las raíces (PFR). También se evaluó, el rendimiento (RF), diámetro de fruto (DF) y grados Brix (°Brix). Se encotraron los mayores valores en FN, AF, PSB, RF y DF, en las plantas que recibieron 6.0 mM como NH_4^+; sin embargo, la concentración de N alteró los efectos de la proporción NH_4^+:NO_3^- y a 0.6 mM N, solo RF y DF mantuvieron la tendencia inicial registrada. La concentración de N modifica el efecto de la proporción NH_4^+:NO_3^- y se propone que los efectos de ambos factores sobre la fotosíntesis, el crecimiento y la producción de frutos del arándano estuvieron mediados por su interacción por la disponibilidad de carbohidratos de la planta.

Palabras clave: nitrato; amonio, calidad; rendimiento; carbohidratos.

INTRODUCTION

Nitrogen (N) is considered the most important mineral nutrient for plants, it is found in a higher proportion than other essential elements (1 % to 3 % dry matter), depending on the plant species, phenological stage, and the organ. It is part of fundamental molecules for growth and development such as nucleic acids, amino acids, proteins, chlorophylls and alkaloids (Marschner, 2011). Plants have developed physiological mechanisms to uptake mineral N from the soil, mainly in the forms of nitrate (NO_3^-) or ammonium (NH_4^+) (Errebhi and Wilcox, 1990; Cárdenas-Navarro *et al*., 2006; Lobit *et al*., 2007). The N absorbed as NO_3^- is reduced to NH_4^+ by the enzymes nitrate reductase (NR) and nitrite reductase (NiR). When this process takes place in the leaves, as in most plants, it depends on reductant compounds generated by photosynthesis, and when it takes place in the roots, the reductants compounds are provided by respiration (Scheurwater *et al*., 2002; Li *et al*., 2013). The NH_4^+ absorbed, or previously produced by the reduction of NO_3^- is assimilated directly into amino acids by the enzyme glutamine synthetase (GS) and requires C compounds to get C skeletons and energy, which can come from previously stored carbohydrates or directly from photosynthesis (Li *et al*., 2013; Doyle *et al*., 2021). On the one hand, N deficiency directly and negatively affects photosynthesis reducing stomatal conductance, content of light harvesting proteins and content and/or activity of photosynthetic enzymes (Mu and Chen, 2021) and indirectly, due to photoassimilates accumulation, through a feedback downregulation mechanism (Araya *et al*., 2010). On the other hand, N assimilation consumes carbohydrates originally produced

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by photosynthesis and competes with other physiological processes associated with plant growth and development for these compounds (Li *et al*., 2013).

Plants preferences for NH_4^+ or NO_3^- depend on the species, phenological stage, habitat of species origin and conditions of roots environment such as pH, aeration, temperature (Li *et al*., 2013). *V. corymbosum* L. is native from the temperate forests of North America and grows in the undergrowth, where low temperatures limit the mineralization of the plenty organic matter; in soils with low salinity, acidic pH, abundant organic forms of N, as amino acids and proteins and scarce mineral N, which is mainly found as NH_4^+ (Korcak, 1989; Rosen *et al*., 1990; Metcalfe *et al*., 2011). Therefore, this plant species has evolutionarily adapted to grow in soils with low N availability (Banados *et al*., 2012; Vargas and Bryla, 2015) and to prefer NH_4^+ instead NO_3^-, which allows it to conserve energy, which is an advantage in a low-temperature environment, since the processes of absorption and assimilation of NH_4^+:NO_3^- require less energy than those of NO_3^-; in addition, the assimilation of NO_3^- is restricted by the low content of NR in its leaves and roots (Poonnachit and Darnell, 2004; Osorio *et al*., 2020; Doyle *et al*., 2021; Leal-Ayala *et al*., 2021). Indeed, it has been documented that when N is supplied as NH_4^+, blueberry plants show greater net photosynthesis, leaf area, biomass and content of chlorophyll and mineral nutrients, than when N is provided only as NO_3^- (Osorio *et al*., 2020; Leal-Ayala *et al*., 2021; Yuan-Yuan *et al*., 2021). However, it is unknown if these effects are kept when N availability in the root medium is variable and if there is interaction between the concentration and the ionic form of this element. Therefore, the aim of this work was to study the main effects and the interaction of N concentration and NH_4^+:NO_3^- proportion in the nutrient solution, on net photosynthesis, plant growth, fruit production and quality of blueberry plants var. Biloxi grown in hydroponics.

MATERIAL AND METHODS

Establishment and experimental conditions

An experiment was developed using 96 blueberry plants (*V. corymbosum* L.) var. Biloxi, produced in vitro and hardened in a greenhouse. The 0.3 m high plants with root ball were transplanted in 7.0 L plastic pots containing a substrate composed with a mixture of volcanic gravel "tezontle" (0.005 - 0.007 m diameter) and river sand at 1:2 (v/v) ratio, which was previously disinfected with a 10 % sodium hypochlorite solution. The pots were established in a tunnel-type greenhouse covered with plastic (30 % shading, 7 mil) and located at the Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Michoacán, México (Lat. 19°46'11.3 "N, Long. 101°09'00.1 "O; 1860 m.s.n.m.). During the experiment, the average daily temperature inside the greenhouse was 27 °C and the average daily relative humidity was 47 %.

Experimental management

Throughout the first 22 d after transfer to the greenhouse, all plants received 0.3 L of demineralized water every 12 h (at 8:30 a.m. and 8:30 p.m.), using an automated drip irrigation system. In order to homogenize the plant material, three days after transfer to the greenhouse, thinning was carried out to leave a single stem per plant. Seven days later, the apical part of the stem was cut, leaving ten viable buds, and seven days later the buds and shoots were removed, leaving only one shoot per plant, which was supported with plastic stakes during its growth.

Experimental design

The experiment was bi-factorial and the studied factors were the N concentration and the NH_4^+:NO_3^- proportion in the irrigation solution. The first factor had two levels: 0.6 mM and 6.0 mM, and the second factor had three levels: 100 % NH_4^+, 50 % NH_4^+ : 50 % NO_3^- and 100 % NO_3^-. The combination of these factors produced six treatments, repeated four times resulting in 24 experimental units (EU), which were integrated with four plants each and randomly distributed. The treatments application began on day 22 after plant transfer to the greenhouse. Six irrigation solutions (one per treatment) were prepared based on nutrient solution proposed by Cárdenas-Navarro *et al.* (1998). All solutions were prepared with demineralized water (pH 5.0, electrical conductivity 1.80 dSm^{-1}, $H_2PO_4^-$ concentration 1 Eq m^{-3} and anions and cations balance 16 Eq m^{-3}). In order to maintain this balance, NO_3^- concentrations variations were compensated by varying SO_4^{2-} concentrations, and NH_4^+ concentrations changes were balanced by varying the concentrations of K^+, Ca^{++} and Mg^{++}, always keeping the proportion 25 %, 50 %, 25 %, respectively. Microelements concentrations were as follows: H_3BO_3, 42.0 µM; $CuSO_4.5H_2O$, 1.0 µM; Fe-EDTA, 15.0 µM; $MnSO_4.H_2O$, 23.0 µM; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.3 µM and, 6.0 µM.

Variables evaluated

At fruit production phase, 242 d after treatments application (DAT), net photosynthesis (PN) was measured by EU, using a portable photosynthesis system (Li -6400; LI-COR). The measurement was carried out inside the greenhouse between 7:00 - 9:00 am, on a fully expanded mature leaf randomly selected, keeping 1,000 µmol $m^{-2}s^{-1}$ light intensity, 400 µmol mol^{-1} CO_2 concentration and 25 ºC temperature. A day after, 243 DAT, one plant per EU was taken and organs dissected, to determine: leaf area (LA) with a digital planimeter (LICOR, LI-3100C) and with a precision balance (Mettler Toledo, PR8002) shoots fresh weight (SFW) and roots fresh weight (RFW). The fruit yield (FY) was evaluated on one randomly selected plant (from the beginning of the experiment) per EU and four harvests were make (every 15 d) along the production phase. The fruits of each plant were weighed (Mettler Toledo, PR8002), its equatorial diameter was measured with a digital vernier (Truper, 14388) and, in a representative sample, the total soluble solids content (°Brix) was determined with a digital refractometer (HI, 96801).

Statistical analysis

The data were subjected to a two-ways analysis of variance (ANOVA) and when significant statistical differences were found, the Tukey mean test ($P \leq 0.05$) was applied, using SAS® OnDemand for Academics for Macintosh.

RESULTS

The obtained results on PN showed statistically significant effects of N concentration, NH_4^+:NO_3^- proportion and interaction of both factors (Table 1). The main effects observed is that plants irrigated with 0.6 mM N and those that received this element only as NO_3^- showed lower rate of net CO_2 assimilation (Fig. 1A, 1B). However, when examining factors interaction, it's clear that the effect of the NH_4^+:NO_3^- proportion is different in the two N concentrations studied. The PN drop observed with the increase of NO_3^- proportion in the irrigation solution was only showed by plants that received 6.0 mM of N, while in plants irrigated with 0.6 mM of N non statistically significant differences were detected between NH_4^+:NO_3^- proportion treatments (Fig. 1C).

The plant biomass production analysis revealed that the variables representing the aerial part growth of plant (the LA and the SFW), showed a similar response that PN. In both variables the effects of the studied factors and their interaction were statistically significant (Table 1), thus, the plants irrigated with 0.6 mM N and those receiving only NO_3^- showed the lowest values (Fig. 2A, 2B, 3A, 3B). Nevertheless, the reduction of LA and SFW observed in plants receiving just NO_3^- in the nutrient solution was only perceived in plants irrigated with 6.0 mM N, whereas non statistically significant differences were detected between NH_4^+:NO_3^- proportion treatments when plants were irrigated with 0.6 mM N (Fig. 2C, 3C). On the other hand, the RFW did not showed statistically significant effects associated with the studied factors or its interaction (Table 1, Fig. 4A, 4B, 4C).

When examining fruits production and quality it was observed that FY showed statistically significant effects associated to N concentration, to NH_4^+:NO_3^- proportion and to interaction of both factors (Table 1). The main effects analysis revealed that FY was higher in plants that received solutions with 6.0 mM N (Fig. 5A) and decreased as the proportion of NO_3^- in the irrigation solution increased (Fig. 5B). However, the factors interaction analysis showed that the effect of

Table 1. P values from bi-factorial ANOVA analysis of: net photosynthesis (PN), leaf area (LA), shoots fresh weight (SFW), roots fresh weight (RFW), fruit yield (FY), fruit diameter (FD) and degrees brix (ºBrix). The factors were N concentration in irrigation solution ([N]) (0.6 mM and 6.0 mM), and NH_4^+:NO_3^- proportion in irrigation solution (NH_4^+:NO_3^-) (100 %:0 %, 50 %:50 %, 0 %:100 %); [N] * NH_4^+:NO_3^- indicates the interaction of both factors.

Tabla 1. Valores de P del análisis bifactorial ANOVA de: fotosíntesis neta (FN), área foliar (AF), peso fresco de tallo (PFT), peso fresco de raíz (PFR), producción de frutos (PF), diámetro de fruto (DF) y grados Brix (ºBrix). Los factores fueron concentración de N en la solución de riego ([N]) (0.6 mM and 6.0 mM) y la proporción de NH_4^+:NO_3^- en la solución de riego (NH_4^+:NO_3^-) (100 %:0 %, 50 %:50 %, 0 %:100 %), [N] * NH_4^+:NO_3^- indica la interacción entre factores.

Factor	PN	LA	SFW	RFW	FY	FD	°BRIX
[N]	0.018	0.002	0.009	0.660	<0.0001	0.064	0.189
NH_4^+ : NO_3^-	0.001	0.001	0.000	0.356	<0.0001	<0.0001	0.132
[N] * NH_4^+ : NO_3^-	0.054	0.018	0.035	0.356	0.004	0.320	0.623

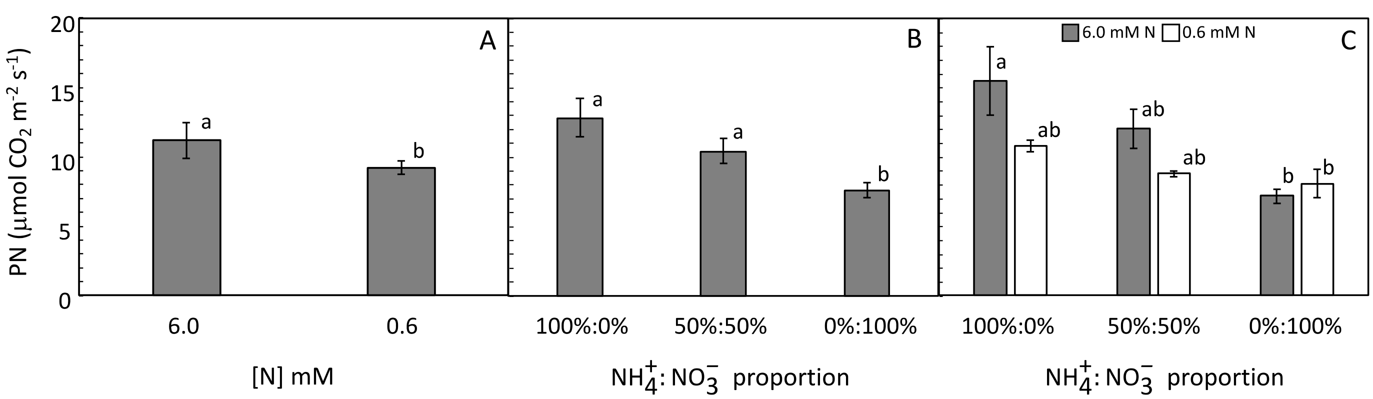

Figure 1. Effect of N concentration in irrigation solution (A); NH_4^+:NO_3^- proportion in irrigation solution (B) and interaction of both factors (C), grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration on net photosynthesis (PN). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at $P \leq 0.05$.

Figura 1. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en la fotosíntesis neta (FN). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una $P \leq 0.05$.

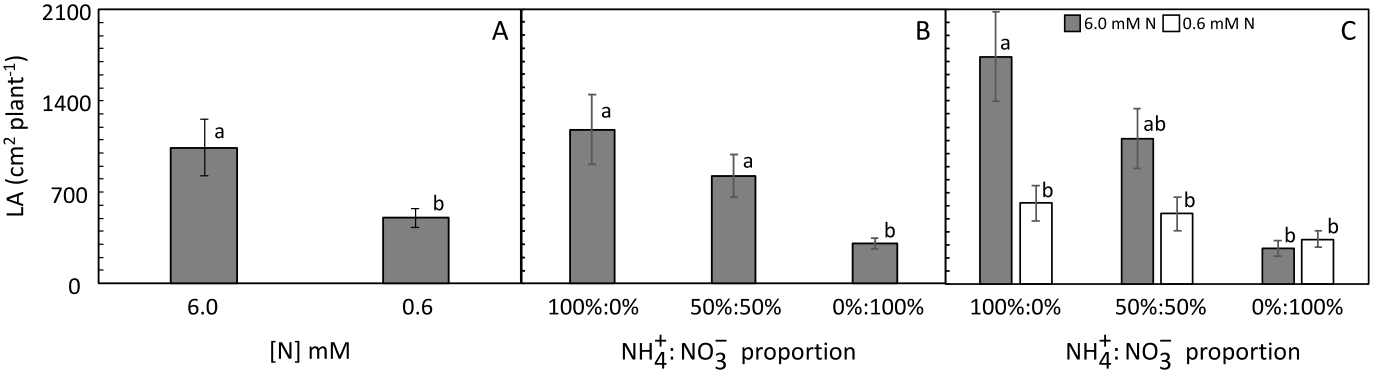

Figure 2. Effect of N concentration in irrigation solution, (A); NH_4^+:NO_3^- proportion in irrigation solution, (B) and interaction of both factors, grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration (C) on leaf area (LA). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at P ≤ 0.05.

Figura 2. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en el área foliar (AF). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una P ≤ 0.05.

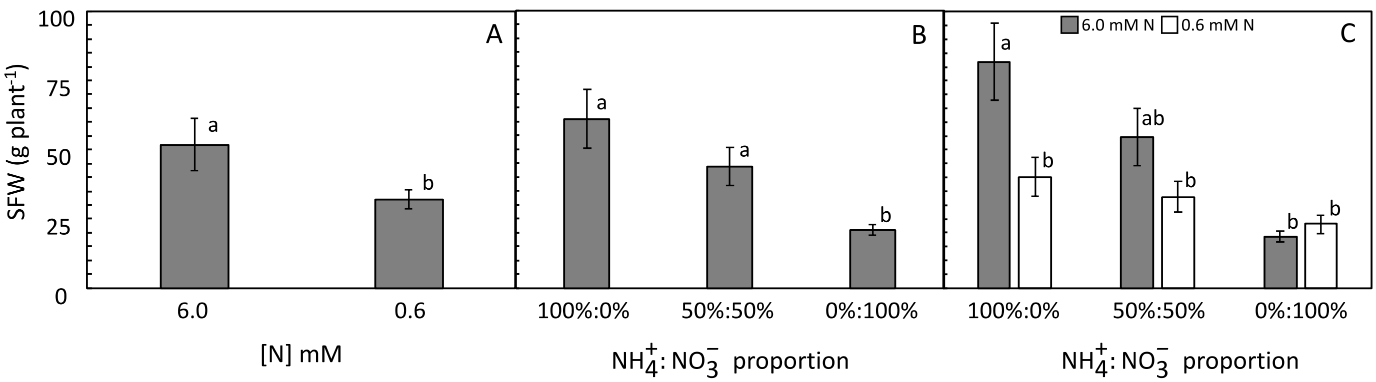

Figure 3. Effect of N concentration in irrigation solution, (A); NH_4^+:NO_3^- proportion in irrigation solution, (B) and interaction of both factors, grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration (C) on shoots fresh weight (SFW). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at *P* ≤ 0.05.

Figura 3. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en el peso fresco de tallos (PFT). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una P ≤ 0.05.

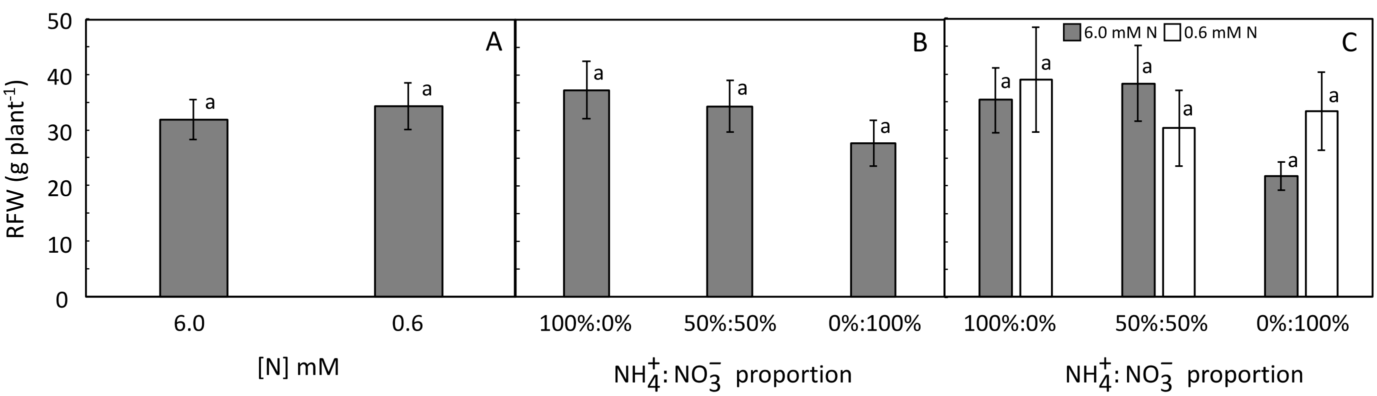

Figure 4. Effect of N concentration in irrigation solution (A); NH_4^+:NO_3^- proportion in irrigation solution (B) and interaction of both factors, grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration (C) on roots fresh weight (RFW). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at P ≤ 0.05.

Figura 4. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en el peso fresco de raíces (PFR). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una P ≤ 0.05.

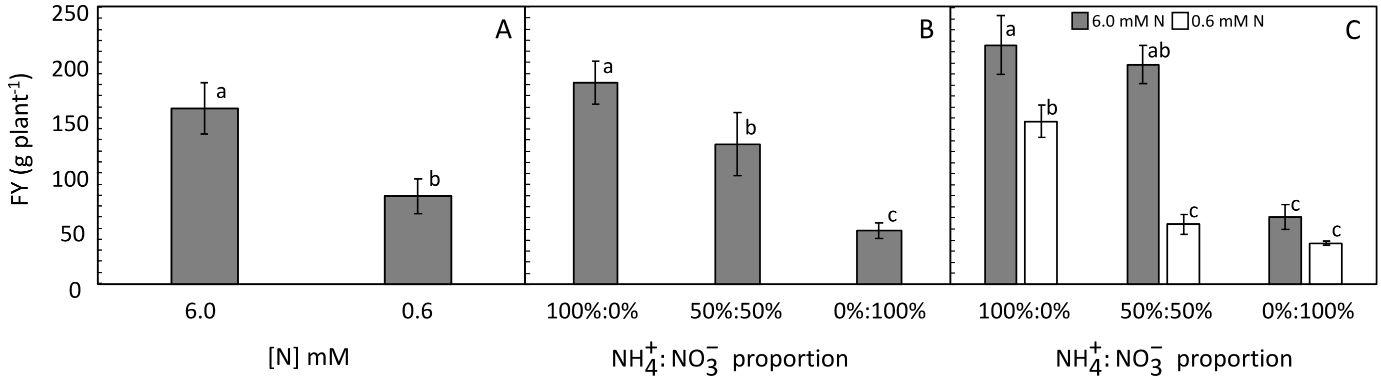

Figure 5. Effect of N concentration in irrigation solution (A); NH_4^+:NO_3^- proportion in irrigation solution (B) and interaction of both factors, grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration (C) on fruit yield (FY). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at P ≤ 0.05.
Figura 5. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en la producción de frutos (PF). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una P ≤ 0.05.

NH_4^+:NO_3^- proportion is different in the two studied concentrations of this element. The plants that received 6.0 mM N had lower FY when this element was provided only in the form of NO_3^-, while in the plants that received 0.6 mM of N, the lower fruits production was observed in treatments with 100 % and also 50 % NO_3^- (Fig. 5C). The FD only showed significant effects associated to NH_4^+:NO_3^- proportion, but not to N concentration or to interaction of both factors (Table 1). The main effects analysis reveals that plants that received only NO_3^- produced the smallest diameter fruits (Fig. 6B) and as the interaction of the studied factors was not significant, this pattern was replicated in both studied N concentrations (Fig. 6C). The ºBrix of fruits did not shows statistically significant effects associated with N concentration, NH_4^+:NO_3^- proportion, or the interaction of both factors (Table 1, Fig. 7A, 7B, 7C).

DISCUSSION

Blueberry is considered a plant with low nutritional requirements, particularly N (Banados *et al*., 2012; Vargas and Bryla, 2015), however, in this work plants that received 0.6 mM N in the irrigation solution showed a lower PN (Fig. 1A). The photosynthesis drop in N-deficient plants is produced by a reduced stomatal conductance, a low availability of energy molecules and light harvesting proteins, as well as reduced content and/or low activity of enzymes involved in carboxylation process (Mu and Chen, 2021). Low photosynthesis in N-deficient blueberry plant is also associated with feedback downregulation mechanism generated by leaf carbohydrates accumulation (Araya *et al*., 2010; Jorquera-Fontena *et al*., 2018). In addition to display a decline in PN, plants that received 0.6 mM N also exhibited less development of the plant aerial part, specifically LA and SFW (Figs. 1A, 2A); therefore, it is evident that

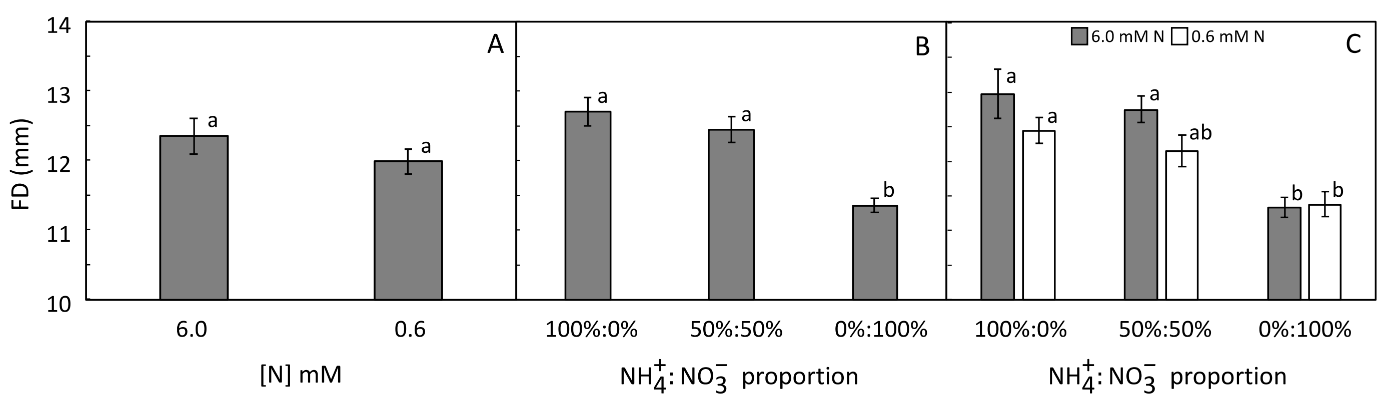

Figure 6. Effect of N concentration in irrigation solution (A); NH_4^+:NO_3^- proportion in irrigation solution (B) and interaction of both factors, grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration (C) on fruit diameter (FD). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at P ≤ 0.05.
Figura 6. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en el diámetro del fruto (D). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una P ≤ 0.05.

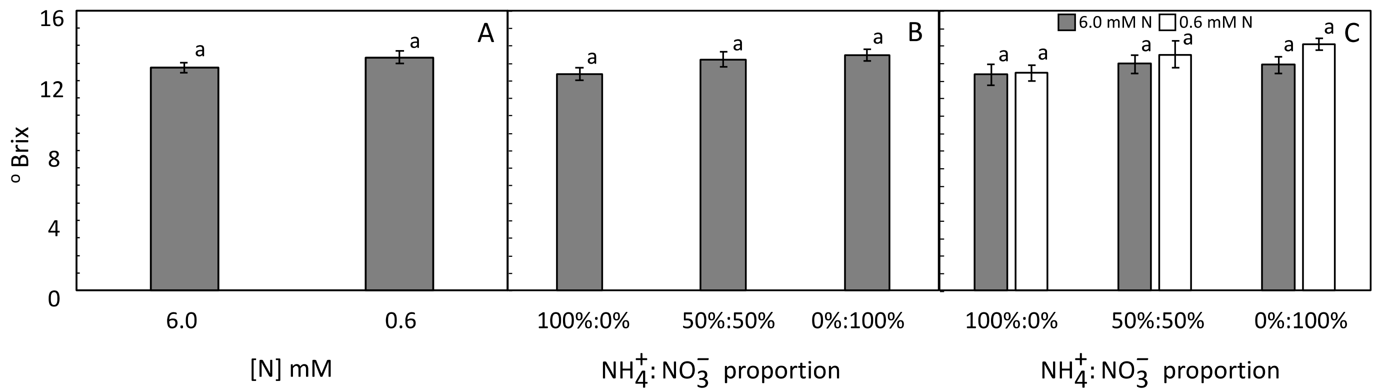

Figure 7. Effect of N concentration in irrigation solution(A); NH_4^+:NO_3^- proportion in irrigation solution (B) and interaction of both factors, grey bars 6.0 mM N concentration and white bars 0.6 mM N concentration (C) on degrees brix (ºBrix). Values are the mean ± standard error; different letters in the bars indicate statistically significant differences according with Tukey test at P ≤ 0.05.
Figura 7. (A). Efecto de la concentracion de N en la solución de riego; (B), proporción de NH_4^+:NO_3^- en la solución de riego y (C), interacción de factores, barras grises concentración de N a 6.0 mM y barras blancas concentration de N a 0.6 mM en los grados Brix (ºBrix). Los valores representan la media ± el error estandar; diferentes letras en las barras indican diferencia estadística significativa de acuerdo con la prueba de Tukey a una P ≤ 0.05.

such a N concentration in the irrigation solution generates a deficiency of this element, which is insufficient to meet the N demand created by plant growth. Moreover, plants that received solutions with 6.0 mM N, together with higher PN, LA and SFW, also showed higher FY (Fig. 5A, 6A, 7A). In most crops, growth and fruit yield rise with the increase of N rate until to a critical level beyond which more N supply increases do not generate augmentations in these variables and may even decline (Cárdenas-Navarro *et al*., 2004). In blueberry several works, carried out under different agronomic conditions, have reported N rates, varying between 34 and 93 kg of N $ha^{-1}year^{-1}$, beyond which increases in plant growth and/or fruit yield were not observed (Bryla and Machado, 2011; Banados *et al*., 2012; Ehret *et al*., 2014; Vargas and Bryla, 2015; Messiga *et al*., 2018). In accordance with these reports, the 6.0 mM N treatments of this work were equivalent to 18.4 g $plant^{-1}year^{-1}$, meaning 51 Kg N $ha^{-1}year^{-1}$ if a common planting density of 2,777 plants ha^{-1} is considered (Banados *et al*., 2012; Vargas and Bryla, 2015). Some reports in the literature indicate that in blueberry N supply reduce the fruits size and affect its sugars accumulation (Ehret *et al*., 2014; Vargas and Bryla, 2015; Zhang *et al*., 2023); however, in this work N treatments did not generate statistical differences neither in FD (Fig. 6A) nor in ºB (Fig. 7A).

Plants uptake mineral N from the soil mainly as NH_4^+ or NO_3^-, the preference between these two ionic forms of N depends on plant species, its origin habitat, its phenological stage and the roots environmental conditions such as pH, temperature, aeration and microbial activity (Li *et al*., 2013). In the literature, most authors claim that blueberry develops better when N is provided only or mainly as NH_4^+ (Doyle *et al*., 2021). In this work, such a preference is confirmed according to the responses registered in the evaluated variables. Firstly, plants that received nutrient solutions in the presence of NH_4^+ showed higher PN (Fig. 1B), compared to those that received only NO_3^-, which agrees with previously published reports on this crop (Osorio *et al*., 2020; Yuan-Yuan *et al*., 2021). Secondly, as is well known, photosynthesis is the main physiological process associated to biomass accumulation and crops production; in this work, plants that received nutrient solutions with NH_4^+, in addition to display higher PN also produced a higher aerial biomass: LA and SFW (Fig. 2B). These results are in accordance with a wide number of previously published reports on blueberry, in which plants biomass increase when supplied with NH_4^+, alone or predominantly, and decrease when received only NO_3^- (Osorio *et al*., 2020; Doyle *et al*., 2021; Yuan-Yuan *et al*., 2021). This behavior is surely associated with *V. corymbosum* L. adaptation to its origin habitat, which is the temperate forests undergrowth and soils with acidic pH, low temperatures, low microbial activity, slow process of organic matter mineralization and NH_4^+ as the predominant inorganic form of N (Korcak, 1989; Metcalfe *et al*., 2011).

The blueberry plant NH_4^+ preference reflects its adaptation to such environmental conditions and is expressed in physiological process as absorption, translocation from the root to the aerial part and assimilation in organic N (Doyle *et al*., 2021). Thirdly, NH_4^+:NO_3^- proportion effects on PN, LA and SFW were likewise observed on the production and quality of fruits. The FY of plants that received only NH_4^+ was threefold than those received only NO_3^- (Fig. 5B), these plants also showed bigger FD (Fig. 6B), although non-statistical differences were observed in ºBrix (Fig. 7B), which agrees with previous reports (Bolaños-Alcántara *et al*., 2019). The increases of FY and FD are surely associated with the higher availability of photo-assimilates, as a result of PN increase; the greater development of LA and the higher number of flower buds and consequently of fruits, as outcome of SFW increase of plants that received NH_4^+ in the irrigation solution.

However, the analysis of studied factors interaction showed that the effect of NH_4^+:NO_3^- proportion on PN, LA, SFW and FY depends on N availability in roots environment. PN of plants that received irrigation solutions with 6.0 mM N was more than twice higher when this element was supplied as NH_4^+, than when it was provided as NO_3^-. In plants that received 0.6 mM N, no statistical differences were observed between NH_4^+:NO_3^- proportion treatments (Fig. 1C). It is well established that N is essential in plants photosynthesis and that when its availability in the root milieu is limited, as is the case of plants that received 0.6 mM N in this work, CO_2 fixation decrease, regardless of the ionic form of N supplied in the irrigation solution. In such a condition, as it has been previously reported by several authors in blueberries, photosynthesis decline, either due to the lack of essential compounds that participate both in the transformation of light energy into energetic molecules and in the carboxylation process, or by the feedback downregulation mechanism exerted by the excessive accumulation of carbohydrates (Araya *et al*., 2010; Jorquera-Fontena *et al*., 2018; Petridis *et al*., 2020; Leal-Ayala *et al*., 2021; Mu and Chen, 2021). On the other hand, when plant growth is not limited by the N availability in the root medium, as in the case of 6.0 mM N treatments, the development of the photosynthetic process is not obstructed by the lack of N compounds nor downregulated by carbohydrates accumulation in leaves cells. In such conditions, PN showed by plants that received only NH_4^+ was more than two folds greater than by those supplied only with NO_3^- (Fig. 1C), which could be associated to carbohydrates consumption by NH_4^+ assimilation processes (Li *et al*., 2013), stimulating CO_2 fixation, according to the sugar-sensing mechanism previously proposed in blueberry (Jorquera-Fontena *et al*., 2018; Petridis *et al*., 2020).

The pattern observed on PN, was similar to plant aerial biomass production both in LA and in SFW. In these variables, plants irrigated with solutions at 6.0 mM N showed six- and five-times greater values, respectively, when N was supplied only as NH_4^+ than when it was provided just as NO_3^- (Fig. 2C, 3C). Furthermore, when the N concentration in the irrigation solution was 0.6 mM none of these variables exposed statistically significant differences between NH_4^+:NO_3^- proportion treatments (Fig. 2C, 3C). These results are surely associated with the previously shown behavior of PN, as this physiological process is considered the most important for plant biomass accumulation (Evans and Clarke, 2019; Flood *et al*., 2011; Yamori, 2020); although probably there are also interactions between the factors and variables studied in this work, with other physiological processes as respiration, water absorption and transpiration.

In blueberry, fruit production depends mainly on the number of plant floral buds (Salvo *et al*., 2011; 2012; Kumarihami *et al*., 2021), whose induction is stimulated by factors such as temperature, light intensity and carbohydrates availability (Pescie *et al*., 2011; Salvo *et al*., 2012; Kumarihami *et al*., 2021). Carbohydrates metabolism plays a fundamental role, since it is a source of soluble sugars such as glucose and fructose, which act as energy providers and as signaling molecules that stimulate the metabolic processes involved in breaking bud dormancy (Wang *et al*., 2021). The first phases of the flower buds induction are sustained mainly by the reserve carbohydrates, and later by fruits photosynthesis, but fundamentally by leaves photosynthesis (Maust *et al*., 1999 a; b). In this context, the production and quality of the fruits is based on a source (leaves) sink (fruit) relationship, whose balance depends fundamentally on the factors that affect the photosynthetic activity of the leaves and the number of fruits (Jorquera-Fontena *et al*., 2018; Kumarihami *et al*., 2021). As previously showed, plants that received 6.0 mM N as NH_4^+, displayed the highest values of PN per unit of leaf surface and in plants that received 0.6 mM N there were no statistically differences between NH_4^+:NO_3^- proportion treatments (Fig. 1C). The same pattern was observed in LA (Fig. 2C) and in SFW (Fig. 3C), therefore, according to the source-sink approach, it can be assumed that the behavior of LA and SFW was associated with photoassimilates availability.

FY and FD showed similar effects than PN, LA and SFW; plants that received 6.0 mM N as NH_4^+ were more than three times and 15 % higher, respectively, than those that received it as NO_3^- (Fig. 5C, 6C); therefore, it could be assumed that the effects showed by FY and FD depends likewise on carbohydrates availability (Maust *et al*., 1999 a; b). However, interestingly and in contrast to PN, LA and SFW, plants that received 0.6 mM N did show statistically significant differences between NH_4^+:NO_3^- proportion treatments in both variables. The FY and FD of plants that received only NH_4^+ were four times and 9.5 % higher, respectively, than those that received only NO_3^- (Fig. 5C, 6C). These results could be explained by an indirect effect of NH_4^+ on flower buds induction and fruit development, through a stimulus to the photosynthetic activity and the increase of photoassimilates availability, which may act as energy providers and/or signaling molecules (Li *et al*., 2013; Jorquera-Fontena *et al*., 2018; Maust *et al*., 1999 a; 1999b; Petridis *et al*., 2020; Wang *et al*., 2021). However, more research is needed to clarify and quantify this process.

CONCLUSIONS

The results of this work show that, in blueberry grown under hydroponic conditions, plant growth and fruit production were higher when N was supplied as NH_4^+, than when it was provided only as NO_3^-. These effects were manifested through higher values of net photosynthesis, leaf area, shoots fresh weight, fruit yield and fruit diameter. However, N concentration in the irrigation solution altered the effect of its ionic form and this pattern was only observed at high N concentration (6.0 mM), whereas at low N concentration (0.6 mM), these effects were only maintained in fruit yield and fruit diameter, but not in net photosynthesis, leaf area and shoots fresh weight. Considering that photosynthesis is a fundamental physiological process for plant growth and development and the intimate relationship between N and C metabolism, it is proposed that the effects of the concentration and the ionic form of N, on growth and fruit production of blueberry plants, were closely related to internal carbohydrates availability.

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CONFLICTS OF INTEREST

The authors will not declare conflicts of interest.

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