

Nitrogen form and root division modifies the nutrimental and biomolecules concentration in blueberry (*Vaccinium corymbosum* L.)

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Abstract

Blueberry (*Vaccinium corymbosum* L.) continues to gain importance in the international market due to its effects on the prevention of human diseases. This leads to the need to optimize the production and quality of the fruit. The present research evaluated the effect of NO_3^- and NH_4^+ , using the split roots technique, in the nutritional status, photosynthetic pigments and total sugars in blueberry leaves. A completely random experiment was established with six greenhouse treatments: three under homogeneous root conduction (HR) and three with split roots (SR). The concentration of N, P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, B and Na, chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car) and total sugars were evaluated in the leaves. The exclusive supply of NH_4^+ led to the largest accumulation of N, P, Mg, S, Cu, Mn and B, compared to plants treated with NO_3^- . The Chla and total sugars were higher with NH_4^+ compared to NO_3^- nutrition. The supply of N separately (SR) had no positive effects on the evaluated variables, however, the SR with half of N, in the form of NH_4^+ , compared to the non-SR with full application of N, has no differences in N-leaf concentration, which implies a higher use in the uptake or accumulation of this macro element in plant. *V. corymbosum* L. with split root and half of N in the form of NH_4^+ , doubled the N use efficiency, as it matches in yield the complete supply treatment of N- NH_4^+ without root division.

Keywords: ammonium; chlorophyll; nitrate; mineral nutrients; split root

Introduction

The blueberry (*Vaccinium corymbosum* L.) is a crop that has gained global economic importance. Its high content of antioxidants contained in the fruits, protect from oxidative damage initiated by free radicals, preventing the development of cardiovascular and neurodegenerative diseases (Howard *et al.*, 2003; You *et al.*, 2011). In Mexico in 2017, the national volume exported reported an all-time high of 26 thousand tons, of which 95.4% was sold to the U. S. market (SIAP, 2018). The study of nutritional requirements allows us to

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make decisions about the production process of the crop and establish a relationship between the nutritional requirement and the quality of the fruit (Castillo *et al.*, 2016). Regarding N, NH_4^+ and NO_3^- represent 80% of the total cations and anions absorbed by plants, because of this, the form of N has a great impact on the uptake of the rest of the elements (Marschner, 2012). Nutritional deficiencies in vegetative-stage crops are reflected in physiological disorders; which subsequently translate into low yields, small sizes, and poor nutritional fruit quality (Mancera *et al.*, 2007; Martínez *et al.*, 2008).

The blueberry (*V. corymbosum* L.), like many calcifuge plants, is adapted to acidic soils (pH 4.0-5.5), with high organic matter content, with iron (Fe) readily available and N in the form of NH_4^+ , relative to N in the form of NO_3^- (Darnell and Hiss, 2006; Bryla *et al.*, 2008). It is well known that NH_4^+ and NO_3^- used separately as a single source of N benefit development and yield according to the species, since generally plants adapted in acidic environments prefer NH_4^+ , otherwise those adapted in alkaline environments prefer NO_3^- (Marschner, 2012).

Plants that absorb NO_3^- require the enzyme nitrate reductase (NR), which is known as inefficient in blueberries (Alt *et al.*, 2017), to reduce NO_3^- to NH_4^+ and metabolize it into amino acids used for protein synthesis (Salisbury and Ross, 2000). González *et al.* (2018) mention that the absorption of NO_3^- by the commercial roots of *Vaccinium* sp. is limited compared to NH_4^+ due to the low activity of the NR (Poonnachit and Darnell, 2004). For the above, the objective of this research was to evaluate the effect of the supply of nitrate (NO_3^-) and ammonium (NH_4^+), by exposing half of the roots to NO_3^- and the other half to NH_4^+ simultaneously, and its effect on physiology of *Vaccinium corymbosum* L.

Materials and Methods

Location

The experiment was conducted in a greenhouse located in the Montecillo Campus of the College of Postgraduate in Agricultural Sciences, State of Mexico, Mexico, whose geographical coordinates are 19° 28' 05" north latitude and 98° 54' 09" longitude west, at an altitude of 2,220 m. The greenhouse used was two-roof with metal structure and UVII-720 plastic cover, with 25x40 anti-insect mesh on the side walls and black cover on the surface of the floor. During the experiment the maximum, average, and minimum temperature was 23.7, 14.6, and 3.0 °C, respectively.

Treatments and experimental design

The experimental unit was a plant of *V. corymbosum* L. with a container consisting of two pots with a flat wall to separate the roots. A completely randomly designed experiment was established with six treatments in a greenhouse condition. Three treatments consisted of a traditional root conduction system by varying only the source of N, the other three treatments consisted of the split root system (SR), with the supply of spatially separated N forms in each pot (Table 1). All treatments were formulated considering Steiner's nutrient solution (Steiner, 1984).

Table 1. Treatments design regarding nitrogen form and root condition

Treatment	N supply form	Concentration ($\text{mol}_{(+)} \text{m}^{-3}$)	Root condition
1	NO_3^- and NH_4^+	6 + 6	^{††} No divided
2	NO_3^-	6 + 6	^{††} No divided
3	NH_4^+	6 + 6	^{††} No divided
4	[†] NO_3^- and NH_4^+	6 / 6	Divided
5	[†] Without N / NO_3^-	0 / 6	Divided
6	[†] Without N / NH_4^+	0 / 6	Divided

[†] = N supply spatially separated (SS). ^{††} = N supply in homogenous media (HM).

Nutrient solution

The composition of Steiner's nutrient solution (1984) was the next ($\text{mol } (+) \text{ m}^{-3}$): 12 NO_3^- , $1 \text{ H}_2\text{PO}_4^-$, 7 SO_4^{2-} , 7 K^+ , 4 Mg^{2+} and 9 Ca^{2+} . The electrical conductivity (EC) of water was 1.0 dS m^{-1} , the pH was adjusted between 4.5 and 5.5 with H_2SO_4 . In addition, the following fertilizers were added as a source of micronutrients: Fe-EDTA, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, H_3BO_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ at a rate of 4.54, 0.43, 0.08, 0.57, 0.016 and 0.02 mg L^{-1} , respectively. A 0.5 HP pump was used for each one of the treatments with self-compensating drippers and watered with 30% drainage in each of the experimental units, with 10 irrigations events per day.

Quantification of nutrients

During the vegetative stage, leaves of *V. corymbosum* L. were collected for drying. Chemical analysis of dry matter was performed for N using the Kjeldhal technique (Watson and Galliher, 2002) and the determination of P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, B and Na using the coupled plasma induction atomic emission spectroscopy equipment (ICP-ES 725, Agilent, Mulgrave, Australia).

Determination of photosynthetic pigments and total sugars

The concentration of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car) were determined according to Casierra *et al.* (2012) and total sugars according to Dubois *et al.* (1956). The leaves of *V. corymbosum* L. stored in liquid N, were macerated and then 60 mg of the tissue were taken and exposed to a triple ethanolic extraction (80, 80 and 50%). In each of the extractions, the samples were placed in a water bath at $80 \text{ }^\circ\text{C}$ for 20 min, then centrifuged to 14 000 rpm during 5 min. The supernatants of each extraction were recovered. The concentration of Chl a, Chl b and Car was determined by reading the extracts at 664, 649 and 470 nm on a spectrophotometer (6715, Jenkway, UK). The concentration of total sugars was determined by weighing 500 mg of fresh tissue already macerated. They were deposited in an Erlenmeyer flask with 50 mL of ethanol 80%. The flasks were placed on a hot iron at constant boiling with occasional agitation until the volume was reduced to 18 mL. The supernatant was filtered and carried to a volume of 20 mL, 1 mL of the extract obtained was taken and 5 mL of anthrone 0.4% (w/v) were added in concentrated H_2SO_4 (Merck KGaA, Darmstadt, Germany). During the process the samples were placed in ice. Afterwards, the samples were incubated in a water bath at $95 \text{ }^\circ\text{C}$ for 15 min; the reaction ended up placing the samples on ice. For quantification, a standard curve was performed using glucose (Sigma-Aldrich, St. Louis Missouri, USA) and the samples were measured at an absorbance of 600 nm on a spectrophotometer (6715, Jenkway, UK).

Statistical analysis

The response variables were subjected to an orthogonal contrast mean test ($p < 0.05$) with the statistical program SAS 9.4. The contrasts of interest are: I ($\text{NH}_4^+ + \text{NO}_3^-$ vs. combined, T3 and T2 vs. T1), II (NH_4^+ vs. NO_3^- , T3 vs. T2), III (SS (NO_3^- and NH_4^+) vs. HM (T4 vs. T1, T2 and T3), IV (SS (Without N/ NH_4^+) vs. HM (T6 vs. T1, T2 and T3) and V (SS (without N/ NO_3^-) vs. HM (T5 vs. T1, T2 and T3). In the following tables it will use I, II, III IV and V to brief contrast.

Results and Discussion

Ammonium (NH_4^+) and nitrate (NO_3^-) as exclusive supply of N

The exclusive supply of NH_4^+ as a source of N had significant effects ($p \leq 0.01$) on the concentration of N, P, Mg, S, Cu, Mn and B, compared to plants treated with NO_3^- (Tables 2 and 3). N concentrations in all treatments ranged from 1.8 to 2.6%, which fall into the sufficiency ranges for blueberry (*V. corymbosum* L.) (Hart *et al.*, 2006). The preference of blueberry for NH_4^+ has been associated with a generally low ability to assimilate the form of NO_3^- , especially within the bud tissues (Alt *et al.*, 2017). This may be due to the genetic

adaptation of blueberry to acidic environments in which NH_4^+ forms predominate over those of NO_3^- (Marschner, 2012). In addition, the uptake rate of NH_4^+ is much higher than that of NO_3^- (Miller and Hawkins, 2007) and, finally, the NH_4^+ absorbed by plants is rapidly metabolized into organic nitrogen compounds compared to NO_3^- (Darnell and Cruz, 2011; Bryla *et al.*, 2012).

Table 2. Analysis of variance and orthogonal contrasts for the concentration of N, P, K, Ca, Mg and S (g kg^{-1}) in blueberry leaves

Contrast	Nitrogen				Phosphorus			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	1.88	0.0250 *	22.883	21.000	0.02	0.8823 ns	1.945	1.920
II T3 vs. T2	4.63	0.0001 *	25.200	20.567	0.72	0.0026 *	2.307	1.583
III T4 vs. T1, 2 and 3	1.04	0.1581 ns	23.300	22.256	-0.19	0.2236 ns	1.737	1.937
IV T6 vs. T1, 2 and 3	-0.55	0.4389 ns	21.700	22.256	-0.20	0.2095 ns	1.730	1.937
V T5 vs. T1, 2 and 3	-3.68	0.0002 *	18.567	22.256	-0.18	0.2462 ns	1.747	1.937
Contrast	Potassium				Calcium			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	0.77	0.0025 *	5.590	4.820	0.64	0.0029 *	5.212	4.567
II T3 vs. T2	0.09	0.6970 ns	5.637	5.543	0.25	0.2352 ns	5.337	5.087
III T4 vs. T1, 2 and 3	0.27	0.1780 ns	5.607	5.333	-1.30	<.0001*	3.687	4.997
IV T6 vs. T1, 2 and 3	0.29	0.1464 ns	5.630	5.333	-0.48	0.0119 *	4.513	4.997
V T5 vs. T1, 2 and 3	0.78	0.0015 *	6.117	5.333	-0.19	0.2594 ns	4.803	4.997
Contrast	Magnesium				Sulphur			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	0.25	0.0085 *	1.635	1.377	0.96	0.0043 *	3.563	2.603
II T3 vs. T2	0.37	0.0021 *	1.820	1.450	2.92	<.0001 *	5.023	2.103
III T4 vs. T1, 2 and 3	0.13	0.1168 ns	1.680	1.549	0.55	0.0516 ns	3.800	3.243
IV T6 vs. T1, 2 and 3	0.19	0.0298 *	1.740	1.549	0.06	0.8002 ns	3.310	3.243
V T5 vs. T1, 2 and 3	0.13	0.1168 ns	1.680	1.549	-0.50	0.0727 ns	2.737	3.243

Means of the different groupings were compared with a significance level of 5%. Positive values in the estimator (Est) - the first group is greater than the second group. Negative values in the estimator the first group is lower than the second group. ns= not significant ($p > 0.05$), *= significant ($p \leq 0.05$). SS= supply of N separately, SH= traditional and homogeneous supply.

Significant concentration of P coincides with what is reported by Mengel and Kirkby (2001) plants supplied with NH_4^+ , generally contain high concentrations of anions, such as P, favoring their absorption and accumulation to maintain electroneutrality at the cellular level. On the other hand, Jing *et al.* (2010) mention that with the addition of NH_4^+ , decreases the pH of the rhizosphere, thus raising the availability of P and the growth of maize plants. This response is reported in different crops supplied with NH_4^+ (Stratton *et al.*, 2001; Parra *et al.*, 2012).

In Mg concentration, the significant increases in leaves with an exclusive supply of NH_4^+ coincide with what is reported by Crisóstomo *et al.* (2014) who confirm a high accumulation of Mg under nutrition with NH_4^+ in contrast to those of NO_3^- and the mixture of both in cranberry cultivation. According to Marschner (2012), NH_4^+ can inhibit the absorption and accumulation of cations, like Mg, however, this depends on the plant species and growth conditions (Lasa *et al.*, 2000).

As for Ca, treatments had no significant effects (Table 2). This coincides with Parra *et al.* (2012), who had no effect on Ca concentration under different sources of N in blueberry cultivation. Ca is not affected in

blueberry plants probably due to its preference for NH_4^+ . Unlike other crops where Ca uptake is limited by direct competition with NH_4^+ (Mengel and Kirkby, 2001).

On the other hand, no significant effects were obtained for K concentration with the supply of the different forms of N (Table 2). It has been noted that uptake, and therefore the concentration of K in leaves and other plant tissues, depends to a large extent on the level of NH_4^+ , as K^+ is a counter ion of NH_4^+ and it compete to enter the cell (Stratton *et al.*, 2001; Szczerba *et al.*, 2006). The null effect of treatments may be due to the adaptation of blueberry to environments where NH_4^+ forms predominate, in addition when plants meet with an optimal pH range the yield is higher, even when pH is not optimal limited absorption of N does not affect development (Ochmian *et al.*, 2019).

In the specific case of S, the noticeable increase in this element in the exclusive treatment of NH_4^+ it is due to the additional supply of Ca and N based on calcium sulfate and ammonium sulfate to formulate the NO_3^- free treatment.

Regarding micronutrients, the concentration of Cu, Mn and B was significant with an exclusive supply of NH_4^+ compared to that of NO_3^- (Table 3). In the case of Cu, this is due to the positive relationship with the N for mobilization and concentration in leaves (Marschner, 2012). It is presumed that the mobilization and availability of Cu can be controlled by the metabolism of the N, since, this micro element has a high affinity for complexes forms, and in particular, with proteins rich in cysteine and amino acids where Cu would be chelated (Marschner, 2012). This is important since more than 99% of Cu is found in a common way in the roots and sap of xylem (Kumar *et al.*, 2009), similar to the results obtained in this research. The high accumulation of Cu coincides with the increased accumulation of N in the treatment where the supply was exclusive with NH_4^+ , due to the increased absorption and accumulation of N by the genetic adaptation of cranberry (Crisóstomo *et al.*, 2014).

In the case of Mn in leaves, it is consistent with Stratton *et al.* (2001), who reported that the concentration of Mn increases as the concentration of NH_4^+ in the nutrient solution increases. On the other hand, the opposite happens when the concentration of NO_3^- in *Ligustrum ibolium* L. increases. In the same way, Husted *et al.* (2005) found increased accumulation of Mn in barley leaves (*Hordeum vulgare*) fertilized with NH_4^+ , compared to those of NO_3^- . However, the accumulation of this micro element is due to the high concentration of S for the formulation of this treatment, since, S and its different forms has a strong influence on the mobilization and accumulation of Mn in dry matter (Husted *et al.*, 2005). This is consistent with this research, as the highest concentration of S occurred in the exclusive treatment of NH_4^+ .

The concentration of B coincides with findings of Sotiropoulos *et al.* (2003), who despite having no difference in leaves, obtained more B in kiwi root (*Delicious actinidia*), with an exclusive nutrition of NH_4^+ compared to that of NO_3^- . The higher concentration of B using NH_4^+ may be due to the formation of $\text{B}(\text{OH})_4^-$ following the high absorption of anions such as NO_3^- that affect the availability of this micro element (Marschner, 2012). On the other hand, the concentration of Fe and Zn was not affected by the form of N (Table 3), which is the opposite of what Crisóstomo *et al.* (2014) reported, as they found a response in the concentration of Fe in blueberry leaves under nutrition with different forms of N.

Similarly, Darnell and Hiss (2006) state that NO_3^- has no effects on the absorption and assimilation of Fe in two blueberry cultivars (*V. corymbosum* and *V. arboreum*), ensuring that pH in soil or substrate is the main limiting factor in the uptake of Fe. Regarding the Zn, it is consistent with what Bryla *et al.* (2012) obtained. They demonstrated that N has no effect on the accumulation of Zn in blueberry. Hafeez *et al.* (2013) assert that the greatest effect of N forms on the absorption of Zn is due to the change in pH that this cause. However, non-significance differences may be due to that the pH was the same in all treatments in this research.

Table 3. Analysis of variance and orthogonal contrasts for the concentration of Fe, Cu, Zn, Mn and B (mg.kg⁻¹ dry matter) in leaves of blueberry

Contrast	Iron				Copper			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	-4.65	0.554 ns	111.737	116.393	1.01	0.2067 ns	11.358	10.350
II T3 vs. T2	13.44	0.1542 ns	118.460	105.013	6.58	<.0001 *	14.650	8.067
III T4 vs. T1, 2 and 3	-13.06	0.0954 ns	100.223	113.289	-0.69	0.3458 ns	10.323	11.022
IV T6 vs. T1, 2 and 3	-34.76	0.0004 *	78.523	113.289	0.23	0.7511 ns	11.253	11.022
V T5 vs. T1, 2 and 3	1.96	0.7902 ns	115.253	113.289	-1.85	0.0232 *	9.170	11.022
Contrast	Zinc				Manganese			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	1.10	0.3609 ns	24.727	23.627	52.85	0.0840 ns	457.067	404.210
II T3 vs. T2	-2.87	0.0527 ns	23.290	26.163	137.5	0.0011 *	525.843	388.290
III T4 vs. T1, 2 and 3	-3.94	0.0035 *	20.410	24.360	-176.8	<.0001 *	262.623	439.448
IV T6 vs. T1, 2 and 3	-0.83	0.4583 ns	23.523	24.360	-97.1	0.0032 *	342.300	439.448
V T5 vs. T1, 2 and 3	-3.35	0.0097 *	21.007	24.360	-96.1	0.0034 *	343.313	439.448
Contrast	Boron							
	Parameters		Means					
	Est	P>t	L (+1)	R (-1)				
I T3 y T2 vs. T1	8.87	0.0002 *	69.790	60.917				
II T3 vs. T2	18.0	<.0001 *	78.793	60.787				
III T4 vs. T1, 2 and 3	4.76	0.0100 *	71.597	66.832				
IV T6 vs. T1, 2 and 3	0.74	0.6419 ns	67.577	66.832				
V T5 vs. T1, 2 and 3	0.47	0.7647 ns	67.310	66.832				

Means of the different groupings were compared with a significance level of 5%. Positive values in the estimator (Est) - the first group is greater than the second group. Negative values in the estimator the first group is lower than the second group. ns= not significant ($p > 0.05$), * = significant ($p \leq 0.05$). SS= supply of N separately, SH= traditional and homogeneous supply.

Combined nitrogen supply

The supply of nitrate and ammonium did not affect the concentration of leaf nutrients; contrary to when separated, there were significant differences ($p \leq 0.05$) in N, K, Ca, Mg, S (Table 2) and B (Table 3). Combining these two forms of N into one nutrient solution results in lower effects compared to single-source supplies. González *et al.* (2018) assessed the effect of different sources of N on the growth of *Vaccinium meridionale* S. in vegetative stage; they tested four treatments, the N-free control, 50% NH₄⁺, 50% NO₃⁻, 100% N as NH₄⁺ and 100% N in the form of NO₃⁻. The plants that achieved the highest growth were those nourished with NH₄⁺, followed by 50/50 treatment.

On the other hand, Poonnachit and Darnell (2004) found that in the presence of NO₃⁻ blueberry plants absorbed it, but in less quantity than NH₄⁺. Alt *et al.* (2017) observed an increase in the assimilation of NO₃⁻ in the roots, when NO₃⁻ is supplied, suggesting that a large proportion of the NO₃⁻ absorbed was assimilated within this organ. Bryla *et al.* (2015) found that the application of NH₄⁺ + NO₃⁻, had intermediate nutritional levels in leaves, compared to the treatment where only NH₄⁺ was added as N source. González *et al.* (2018) obtained an increase in the dry weight of bud, leaves, and root in blueberry plants, with the fertilization of N in the form of NH₄⁺, compared to plants fertilized with N-NO₃⁻.

In other researches, Darnell and Hiss (2006) and Darnell and Cruz (2011), found that *V. arboreum* S., a wild species, is more tolerant to a high pH and assimilates better N in NO₃⁻-form, compared to *V. corymbosum* L. However, regardless of whether the plant prefers NO₃⁻ as a source of N (e.g. tomato, pepper, eggplant, cucumber, corn, bean, tobacco, etc.) or NH₄⁺ (e.g. rice, blueberry, etc.), almost all reported results

show that plant growth or yield is superior when receiving a mixture of NO_3^- and NH_4^+ , rather than any single source of N, while the appropriate percentage of NH_4^+ and NO_3^- varies by plant species and is generally related to plant physiological adaptations to natural ecosystems (Xu *et al.*, 2001; Dong *et al.*, 2012).

Effect of N form and split roots on photosynthetic pigments

The N-form showed significant effect ($p \leq 0.05$). In the contrast of NH_4^+ against NO_3^- (Table 4): Cla, was higher in plants supplied with NH_4^+ compared to the group supplied with NO_3^- . This is due to a characteristic of blue cranberry (*V. corymbosum* L.) of preferring NH_4^+ as a source of N. In addition, to split the root had no significant effect on Chl *a*, Chl *b* and Chl *t* concentration (Table 4). However, it is important to note that the split root contrast (without N/ NH_4^+) *vs.* no divided root, was not significant. This indicates that half of the N applied in the form of NH_4^+ has similar results compared to a complete application of N- NH_4^+ in no divided root condition (Table 4). According to Latsague *et al.* (2014), the photosynthetic capacity of leaves is related to the N content, because an adequate supplement of N is essential in the establishment of maximum demand capacity and photosynthetic capacity; but excessive use of N causes excessive expansion of the foliar area. Jorquera-Fontena *et al.* (2016) observed that the accumulation of photosynthetic pigments in blueberry plants is an indicator of biomass production and the nutritional status of N. According to most of the research done, the ratio of chlorophyll *a* and *b* is 3:1, which varies depending on plant growth and development, cultivar, and environmental factors (Bojovic and Stojanovic, 2005). Sánchez *et al.* (2018) obtained a positive and significant correlation of the concentration of chlorophyll *a*, chlorophyll *b* and carotenoids with the nutritional state of N, in bean (*Phaseolus vulgaris* L.).

An adequate supply of N in the plant is important for the formation of amino acids, proteins, and other cellular constituents (Trejo-Téllez *et al.*, 2005) and has a positive effect on photosynthesis and respiration (Bar-Tal *et al.*, 2001). Plants with N deficiency stop the elongation of the leaves (Marschner, 2012), inhibit photosynthesis (Gregoriou *et al.*, 2007), reduce the size of chloroplast (Li *et al.*, 2013) and minimize overall growth. Similarly, the content of N is closely related to photosynthetic capacity, since it constitutes chlorophyll, thylakoid proteins and enzymes (Rubisco mainly) (Kitaoka and Koike, 2004; Watanabe *et al.*, 2018). In addition, N tends to promote vegetative growth (Kang *et al.*, 2004), increases the root/shoot ratio of the plant (Grechi *et al.*, 2007).

Nagamatsu *et al.* (2004) mention that plants subjected to any type of stress tend to lose photosynthetic ability and decrease its content in leaves. The level of stress can produce a readjustment in the proportion of the contents of chlorophylls (Chl *a* and Chl *b*), this can be understood as a typical response of photosynthetic plasticity (Habibi and Ajory, 2015). In the case of the results obtained in this research, the application of N in the form of NH_4^+ and NO_3^- , and the use of Split root against entire root, did not cause enough stress in the plants to express significant effect on the Chl *a*, Chl *b*, and Car.

The total sugar concentration in blueberry shoots had significant differences ($p \leq 0.01$) by the application of NH_4^+ or NO_3^- compared to the combined application of these forms of N (Table 4). In addition, significant differences were obtained for the supply of N in the form of NH_4^+ , over the supply of N in the form of NO_3^- , this is consistent with the characteristics presented by *V. corymbosum* L., which is a low pH soil plant, and it has a greater preference for N in the form of ammonium than the N in the form of nitrate (Bryla *et al.*, 2008). Using split root model *vs.* Entire root did not present significant differences in the total sugar content in any of the three contrasts made (Table 4).

The measurement of Chl*a* and Chl*b* is used as an estimator of photosynthesis, its increase stimulates the photosynthetic rate by increasing the content of soluble sugars (Sanclemente and Peña, 2008). Excess sugars can protect sensitive membranes and proteins from dehydration due to cold, drought and salinity. However, they are inaccessible for growth and may instead inhibit photosynthesis and cause an increase in photoassimilation (Paul and Foyer, 2001; Lemoine *et al.*, 2013). According to Araya *et al.* (2010), limited nutrition of N induces accumulation of carbohydrates in the leaves of *Phaseolus vulgaris* L. When nutrition with N is insufficient, the size of the plant's demand is small because the plant's growth is restricted.

In the case of this work, the results of total sugars (Table 4) showed only that there is a significant response due to the source of N supplied, with NH_4^+ inducing the highest amount of total sugars.

Table 4. Analysis of variance and orthogonal contrasts for chlorophyll *a*, *b*, carotenoids and total bud sugars (mg g^{-1}) in vegetative blueberry plants

Contrast	Chlorophyll <i>a</i>				Chlorophyll <i>b</i>			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	-0.01	0.6531 ns	0.208	0.220	-0.02	0.3223 ns	0.111	0.133
II T3 vs. T2	0.08	0.0230 *	0.246	0.170	0.03	0.1987 ns	0.128	0.094
III T4 vs. T1, 2 and 3	0.02	0.3879 ns	0.233	0.212	-0.001	0.9904 ns	0.118	0.118
IV T6 vs. T1, 2 and 3	-0.04	0.1565 ns	0.176	0.212	-0.02	0.2845 ns	0.095	0.118
V T5 vs. T1, 2 and 3	0.01	0.6394 ns	0.224	0.212	-0.01	0.4879 ns	0.103	0.118
Contrast	Carotenoids				Total sugars			
	Parameters		Means		Parameters		Means	
	Est	P>t	L (+1)	R (-1)	Est	P>t	L (+1)	R (-1)
I T3 y T2 vs. T1	0.005	0.5378 ns	0.067	0.062	8.56	0.0181 *	38.150	29.588
II T3 vs. T2	0.018	0.0631 ns	0.076	0.058	9.63	0.0207 *	42.963	33.338
III T4 vs. T1, 2 and 3	-0.001	0.9818 ns	0.065	0.065	-1.58	0.6015 ns	33.713	35.296
IV T6 vs. T1, 2 and 3	-0.008	0.2734 ns	0.057	0.065	6.33	0.0531 ns	41.629	35.296
V T5 vs. T1, 2 and 3	0.007	0.3840 ns	0.072	0.065	4.17	0.1835 ns	39.463	35.296

Means of the different groupings were compared with a significance level of 5%. Positive values in the estimator (Est) - the first group is greater than the second group. Negative values in the estimator the first group is lower than the second group. ns= not significant ($p > 0.05$), *= significant ($p \leq 0.05$). SS= supply of N separately, SH= traditional and homogeneous supply.

Supply of N separately (SS) and homogeneous supply (HS)

The Ca was the only macronutrient that showed significant differences ($p \leq 0.05$) with contrast (NO_3^- and NH_4^+) vs. SH (Table 2), while Zn, Mn, and B micros had significant differences at too in that same contrast (Table 3).

Moreover, the contrast with only half of N in the form of NH_4^+ , had significant differences in Mg, Ca, Mn, and Fe (Table 2 and 4), the rest of the variables were not significant for this contrast. In this case, there was higher use of blueberry plants, with half the concentration of NH_4^+ . This result allows us to obtain a better use of the applied fertilizer, making use of the SS technique, without losses in the production of blueberry. This phenomenon can be attributed to the compensatory function of the roots divided under different stress conditions (Péret *et al.*, 2011; Gao *et al.*, 2013). Zhu and Ito (2000) obtained a higher dry weight of roots and higher concentration of Ca in potato seedlings, in SS with half the nutrient solution that control. Shabnam *et al.* (2018), reported that when SS technique is used in wheat plants in an acid soil it increases the accumulation of P. Even, in trifoliolate orange seedlings where SS was used in combination with mycorrhizal fungi (MF), the presence of MF on one side of the split roots benefited the acquisition of C and the development of the root in the other half without MF (Qian-Sheng *et al.*, 2016).

It has also been reported that under SS the accumulation of dry matter of the root, was 18% lower in the part supplied with NH_4^+ than in that supplied with NO_3^- in plants of *Phaseolus vulgaris* L. (Guo *et al.*, 2007). The SS can suppress growth of the root of tobacco plants when deprived of NH_4^+ in the side of the SS compared to the other party receiving NO_3^- (Walch-Liu *et al.* 2001). Finally, it has been reported that the combined application of NH_4^+ and NO_3^- in tomato seedlings is better for the growth and uptake of N (Dong *et al.*, 2012).

In the last contrast with N-only application in NO_3^- form, significant differences were obtained for N, K, Zn, Mn and Cu (Tables 2 and 3) confirming the tendency of blueberry plant to uptake N in the form of NH_4^+ .

Conclusions

The supply of nitrate to half the root and ammonium to the other half simultaneously improves the physiological development and nutritional status of *Vaccinium corymbosum* L. compared to undivided root plants. The concentration of N, P, Mg, S, Cu, Mn, B, chlorophyll *a* and total sugars in plant leaves nourished with NH_4^+ is higher than that of plants with NO_3^- . Split root plants, without N/ NH_4^+ and half N supply, achieved the same yield as full N supply plants without root division. *V. corymbosum* L. Biloxi variety with split root and half of N in the form of NH_4^+ , doubled the use of N, as it matches in yield the complete supply treatment of N- NH_4^+ without root division.

Authors' Contributions

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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