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 NO₃⁻ and NH₄⁺, 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 NH₄⁺ led to the largest accumulation of N, P, Mg, S, Cu, Mn and B, compared to plants treated with NO₃⁻. The Chl a and total sugars were higher with NH₄⁺ compared to NO₃⁻ 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 NH₄⁺, 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 NH₄⁺, doubled the N use efficiency, as it matches in yield the complete supply treatment of N-NH₄⁺ 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
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).

| Treatment | N supply form | Concentration (mol (+) 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 (mol (+) m⁻³): 12 NO₃⁻, 1 H₂PO₄⁻, 7 SO₄²⁻, 7 K⁺, 4 Mg²⁺ and 9 Ca²⁺. The electrical conductivity (EC) of water was 1.0 dS m⁻¹, the pH was adjusted between 4.5 and 5.5 with H₂SO₄. In addition, the following fertilizers were added as a source of micronutrients: Fe-EDTA, MnSO₄·4H₂O, ZnSO₄·7H₂O, H₃BO₃, CuSO₄·5H₂O and Na₂MoO₄·2H₂O 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 °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 644, 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 H₂SO₄ (Merck KGaA, Darmstadt, Germany). During the process the samples were placed in ice. Afterwards, the samples were incubated in a water bath at 95 °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 (NH₄⁺ + NO₃⁻ vs. combined, T3 and T2 vs. T1), II (NH₄⁺ vs. NO₃, T3 vs. T2), III (SS (NO₃ and NH₄) vs. HM (T4 vs. T1, T2 and T3), IV (SS (Without N/NH₄⁺) vs. HM (T6 vs. T1, T2 and T3) and V (SS (without N/NO₃) 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 (NH₄⁺) and nitrate (NO₃⁻) as exclusive supply of N**

The exclusive supply of NH₄⁺ as a source of N had significant effects (p ≤ 0.01) on the concentration of N, P, Mg, S, Cu, Mn and B, compared to plants treated with NO₃ (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 NH₄⁺ has been associated with a generally low ability to assimilate the form of NO₃, 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 |
| I T3 vs 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 T6 vs T1,2 and 3 | -3.68 0.0002 * | 18.567 22.256 | -0.18 0.2462 ns | 1.745 1.937 |

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| Contrast | Potassium | | Calcium | |
|----------|-----------|----------|-------------|----------|
|          | Parameters | Means | Parameters | Means |
| I T3 vs 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 T6 vs T1,2 and 3 | 0.78 0.0015 * | 6.117 5.333 | -0.19 0.2594 ns | 4.803 4.997 |

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| Contrast | Magnesium | | Sulphur | |
|----------|-----------|----------|-------------|----------|
|          | Parameters | Means | Parameters | Means |
| I T3 vs 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 0.1168 ns | 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 T6 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 B(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 Parameters | Cu Parameters |
|---------------------|-----------------|---------------|
|                     | 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 111.737 111.393 | 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 | -0.15 11.358 10.350 |

| Contrast            | Zn Parameters | Mn Parameters |
|---------------------|---------------|---------------|
|                     | 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            | B Parameters |
|---------------------|---------------|
|                     | 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         | 4.67 <.0001 * 78.793 60.787 |
| III T4 vs T1, 2 and 3 | 0.74 0.6419 ns 67.577 66.832 |
| IV T6 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 ≤ 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 ≤ 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% NH\(_4^+\), 50% NO\(_3^-\), 100% N as NH\(_4^+\) and 100% N in the form of NO\(_3^-\). The plants that achieved the highest growth were those nourished with NH\(_4^+\), followed by 50/50 treatment.

On the other hand, Poonnachit and Darnell (2004) found that in the presence of NO\(_3^-\) blueberry plants absorbed it, but in less quantity than NH\(_4^+\). Alt et al. (2017) observed an increase in the assimilation of NO\(_3^-\) in the roots, when NO\(_3^-\) is supplied, suggesting that a large proportion of the NO\(_3^-\) absorbed was assimilated within this organ. Bryla et al. (2015) found that the application of NH\(_4^+\) + NO\(_3^-\) had intermediate nutritional levels in leaves, compared to the treatment where only NH\(_4^+\) 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 NH\(_4^+\), compared to plants fertilized with N-NO\(_3^-\).

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 NO\(_3^-\)-form, compared to V. corymbosum L. However, regardless of whether the plant prefers NO\(_3^-\) as a source of N (e.g. tomato, pepper, eggplant, cucumber, corn, bean, tobacco, etc.) or NH\(_4^+\) (e.g. rice, blueberry, etc.), almost all reported results
show that plant growth or yield is superior when receiving a mixture of NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+}, rather than any single source of N, while the appropriate percentage of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} 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 ≤ 0.05). In the contrast of NH\textsubscript{4}\textsuperscript{+} against NO\textsubscript{3}\textsuperscript{−} (Table 4): Cla, was higher in plants supplied with NH\textsubscript{4}\textsuperscript{+} compared to the group supplied with NO\textsubscript{3}\textsuperscript{−}. This is due to a characteristic of blue cranberry (V. corymbosum L.) of preferring NH\textsubscript{4}\textsuperscript{+} as a source of N. In addition, to split the root had no significant effect on Chl \textit{a, b} and Chl \textit{c} concentration (Table 4). However, it is important to note that the split root contrast (without N/ NH\textsubscript{4}\textsuperscript{+}) vs no divided root, was not significant. This indicates that half of the N applied in the form of NH\textsubscript{4}\textsuperscript{+} has similar results compared to a complete application of N-NH\textsubscript{4}\textsuperscript{+} 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 \textit{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 \textit{a}, chlorophyll \textit{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 \textit{a and 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\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−}, and the use of Split root against entire root, did not cause enough stress in the plants to express significant effect on the Chl \textit{a, b} and Car.

The total sugar concentration in blueberry shoots had significant differences (p ≤ 0.01) by the application of NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{−} 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\textsubscript{4}\textsuperscript{+}, over the supply of N in the form of NO\textsubscript{3}\textsuperscript{−}; 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 \textit{a} and Chl \textit{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 photoasimilation (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          | Carotenoids Parameters | Means | Total sugars Parameters | Means |
|-------------------|------------------------|-------|-------------------------|-------|
|                   | Est | $P>t$ | L (+1) | R (-1) | Est | $P>t$ | L (+1) | R (-1) |
| I T3 vs T2, 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 | -0.01 | 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 |

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 trifoliate 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$_4^+$. 

8
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.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

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

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