Evaluation of Different Concentrations of Nitrogen for Tomato Seedling Production (Lycopersicon esculentum Mill.)

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Abstract This study was aimed at evaluating the different concentrations of nitrogen for tomato seedling production (Lycopersicon esculentum Mill.), hybrid Loreto. Five concentrations of nitrogen were analyzed: 0, 4, 8, 12 and 16 mEq / L, using as a basis the Steiner nutrient solution. A pilot randomized block design was used with six replications and five treatments. Thirty-five days after sowing, the following variables were analyzed: seedling height, stem diameter, fresh stem weight, fresh leaf weight, leaf area, dry steam weight, dry leaf weight, dry root weight and total nitrogen content. An ANOVA analysis (p < 0.05) with post-hoc Tukey test was performed to compare each treatment variables. The results showed that the increase in the concentration of nitrogen has a positive effect on organ growth. The treatment with the highest values in the morphological variables was 16 mEq / L, which shortened the production time of seedlings ready for transplant.

Keywords Tomato Seedling, Quality of Seedlings, Nitrate Concentration, Steiner Nutrient Solution

1. Introduction

Agriculture currently focuses on the intensive, rational use of all the available resources —soil, seeds, water— and the efficient management of production and logistics to guarantee producers will obtain the best performance at the lowest possible cost. The current trend is to produce seedlings in greenhouses for their subsequent transplantation. Seedling production is the first and most sensitive stage in the production of vegetable species. It is crucial to obtain the best seedlings that, when transplanted, can grow and perform optimally. Seedling growth and nutritional status are directly related to the earliness, yield, size and number of fruits [1-4]. For greenhouse production of seedlings with the desired characteristics, the use of nutrient solutions, the right mix of organic matter in the soil is highly recommended [5].

The substrate must be suitable for germination and mechanical support of seedlings. In addition, depending on its origin and composition, the substrate can provide nutrients, though hardly sufficient to meet demand in the manner and magnitude that seedlings require with the appearance of the first true leaves [3]. To satisfy the nutritional requirements and obtain vigorous and suitable seedlings for transplantation, the continued application of nutrients through a nutrient solution (NS) is recommended [5], since it can favorably modify the morphological characteristics and growth of the seedlings [6].

The use of an NS in early phenological stages can be an effective strategy to facilitate root growth, minimize seedling stress and increase survival [7]. It is well known that an adequate nutritional status of the seedlings before transplantation has beneficial effects on their morphological and physiological characteristics once transplanted. Proper nutrition at the stage of seedling production ensures acceptable crop yields, increasing the proportion of marketable fruit and / or early harvests. On top of this, uniform seedling growth, the highest growth rate in the seedbed, higher quality and a lower seedling mortality rate are achieved after transplantation [8,9].

Nitrogen is a key element to increase yield and quality in horticultural production [9-12]. An adequate supply of nitrogen is associated with appropriate levels of chlorophyll, vigorous plant growth, high photosynthetic activity and proper synthesis of carbohydrates. This increases performance. Regarding the quality of vegetables, if this nutrient is abundantly supplied and growing conditions are favorable, proteins are synthesized and the protoplasm from the synthesis of carbohydrates results in highly hydrated cell protoplasm (i.e. plants with succulent tissues). In contrast, under conditions of nitrogen deficiency, deposits and carbohydrate accumulate in vegetable cells, which thickens and hardens the tissues. This decreases the quality of the product and the survival rate of plants during their physiological development [10,13].

Among the nutrients plants need to ensure optimal supply at the root zone, from the moment the crop is planted until the end of the agricultural cycle, nitrogen is the most difficult to manage in a fertilization system [14,15]. This is because the ionic forms in which this element is found in the soil solution (NO^{3-} and NH_4^+) may be lost due to high mobility or may react with the environment and be lost by denitrification (NO^{3-}) or volatilization (NH_3), thus decreasing the amount of nitrogen available for the crop.

In a study involving ancho chili seedlings (Capsicum annum L.), variety ancho San Luis, it was reported that nitrogen is a nutrient that must be carefully controlled in the NS [16]. The study, which evaluated the concentration of nitrogen and the osmotic potential of the NS, found that the concentration of nitrogen was the most influential factor in the development of ancho chili seedlings. The reported concentrations tested were 0, 2.5, 5, 10 y 15 mol m^{-3} , with the latter resulting in the best seedling development. Another study evaluated the influence of nitrogen on pepper and tomato seedling growth at concentrations of 25, 50, 75 and 100 ppm. It was reported that increasing the concentration of nitrogen from 25 to 100 ppm allowed for a progressive increase in dry weight for both species [17]. Another study involving husk tomato crops (Phisalis ixocarpa) evaluated the effect of five concentrations of nitrate nitrogen (5, 7, 9, 11 and 13 mEq / L) in the NS. It was reported that the highest accumulation of biomass was obtained with the concentration of 9 mEq / L, whereas the highest harvest index was obtained with 13 mEq / L [10].

Although there are some studies related to the fertilization of seedlings from different species, the different nutritional and phenological aspects that help define the most appropriate dose of nitrogen for each seedling species must be analized, particularly in the case of tomato, given high production levels of this crop globally and the lack of information concerning the effect of nitrogen on the seedlings. The purpose of this study was to evaluate the effect of five concentrations of nitrogen (N-NO₃⁻) in greenhouse production of tomato seedlings (Lycopersicon esculentum Mill.).

2. Materials and Methods

2.1. Localization and Type of Greenhouse

The study was conducted in a greenhouse in Amazcala, municipality of El Marquez in the state of Queretaro, Mexico. Amazcala is located at $20^{\circ} 42' 20$ " north and $100^{\circ} 15' 37$ " west, at 1,921m above sea level. The region has a semi-dry weather. The Gothic single span greenhouse measures $56m^2$ (7 x 8m) and has lateral ventilation only $(24m^2)$. The cladding material was a single layer of long-term polyethylene plastic, topped with shading net to prevent high temperatures.

2.2. Biological Material and Containers

Tomato seed was of an indeterminate growth and the seeds were imported from the USA by the seed center in the city of Celaya, Guanajuato. The substrate used for this study was a commercial organic material made of moss sphagnum ("Premier" peat moss) from Canada, with total nitrogen levels of 1.23% and 250 mg kg^{-1} of N-NO₃⁻.

The germination container was a commercial model made of polystyrene with 200 conical cavities, each with a capacity of 25 ml. The dimensions of the container were 36 cm wide, 62cm long and 7cm height (Fig 1).

2.3. Management of Sowing and Germination

The containers were disinfected using an organic solution based on citrus seed extract, with a concentration of 8ml extract in 1L water. The tomato seeds were kept in water for 1 hour before sowing to accelerate the germination process. The organic substrate was watered up to field capacity. Subsequently, the containers were filled with peat moss up to seven eighths of their capacity and a tomato seed was planted at about 0.5cm depth. It was then covered with vermiculite and watered. Finally, all the containers were piled in groups of seven and then covered with a layer of black plastic. All the containers were kept in a germination room at a temperature of about 25°C and 70% relative humidity until the first plants emerged (about 4 days after sowing). When this occurred, all the containers were transferred to the greenhouse for plants to grow. Sowing took place on September 15th.



Figure 1. Typical image of tomato seedling growth (Lycopersicon esculentum Mill.) in commercial containers of polystyrene with 200 conical cavities.

Table 1.	Nutrient	concentration	and elec	tric condu	ctivity ir	n the f	five solu	utions	tested

Nitrogen ^a	NO3 ^{-a}	$H_2PO_4^{-a}$	${\rm SO_4}^{2\text{-}a}$	$K^{+ a}$	Ca ^{2+ a}	Mg^{2+a}	Fe ^b	Cu ^b	Mn ^b	Zn ^b	$B^{\ b}$	CE °
0	0.0	2.5	9.4	6.9	8.4	4.0	1.9	0.5	0.8	0.3	0.4	2.0
4	4.0	2.0	9.9	6.9	8.4	4.0	1.9	0.5	0.8	0.3	0.4	2.0
8	8.0	1.5	10.4	6.9	8.4	4.0	1.9	0.5	0.8	0.3	0.4	2.0
12	12.0	1.0	7.3	6.9	8.4	4.0	1.9	0.5	0.8	0.3	0.4	2.0
16	16.0	1.0	3.3	6.9	8.4	4.0	1.9	0.5	0.8	0.3	0.4	2.0

^a(mEq L⁻¹), ^b(mg L⁻¹) ^c(dS m⁻¹)

2.4. Nutrient Solutions

The basic nutrient solution used was the one reported by Steiner [18]. The water used to prepare the nutrient solution was rainwater with the following chemical characteristics: pH =7.9; EC=0.30 dS m⁻¹; and mEq L⁻¹ ions of $Ca^{2+}=0.59$, $Mg^{2+}=0.02$, $Na^{+}=0.05$, $K^{+}=0.07$, $CO_{3}^{-}=0.08$, $HCO_{3}^{-}=1.63$, $SO_4=0.14$ and N-NO₃⁻⁼=0.1 mg kg⁻¹. In order to complete the nutrient solution, the following substances were used: Calcium nitrate (Ca(NO₃)₂), 15.5% N, 19% Ca; potassium nitrate (KNO₃), 13% N, 38%K; phosphoric acid (H₃PO₄,), 32%; monopotassium phosphate (KH₂PO₄), 23% P, 28% K; potassium sulfate (K₂SO₄), 45% K, 18% S; magnesium sulfate (MgSO₄) 10% Mg, 13% S; magnesium nitrate (MgNO₃), 11% N, 9% Mg; boric acid (H₃BO₃), 17.5% B; iron chelate (Fe, 13%), manganese chelate (Mn, 13%), zinc chelate (Zn, 14%) and copper chelate (Cu, 14%). The chelate was EDTA. The nutrient solutions (nitrogen treatment) had five concentrations of nitrogen (N-NO₃): 0, 4, 8, 12 and 16mEq L⁻¹. These were evaluated to determine which dose of nitrogen produced the best-quality tomato seedlings for transplantation. Electric conductivity was kept constant at 2.0dS m⁻¹. Table 1 shows the final nutrient solutions.

2.5. Measured Variables

The measured variables were seedling height, stem diameter, fresh stem weight, fresh leaf weight, leaf area, dry stem weight, dry leaf weight, dry weight root and total nitrogen content. For each nitrogen treatment and sampling group, these variables were determined 35 days after sowing.

2.5.1. Seedling Height

This variable was measured in centimeters (cm) using a 5m Mark Truper measuring tape, FH-3m (Truper, Taiwan), with an accuracy of 0.01mm, that was placed vertically on the substrate surface. The measurement was taken on the apical meristem.

2.5.2. Stem Diameter

To make the measurement, a stainless hardened 150mm LCD electronic eigital vernier mark (Grainger, USA) was used. The unit of measurement was millimeters (mm). The stem diameter was measured on the main stem of the plant 1cm above the substrate.

2.5.3. Fresh Stem Weight and 2.5.4 Fresh Leaf Weight

From the experimental unit comprising a tray of 200 cavities, a sample of 10 plants from each of the 6 replicates was taken for each of the 5 treatments. These plants were taken randomly from the center of the experimental unit. Afterwards, the leaves and stems were separated from the

plant and weighed separately in the measuring unit. These two variables were measured using a digital electronic balance 1000X HBR 0.01 g with an accuracy of 0.01 mg.

2.5.5. Leaf Area

The leaf area was determined using digital pictures according to the method proposed by Rico-García *et al* [19].

2.5.6. Dry stem weight, 2.5.7 dry leaf weight and 2.5.8 dry root weight

The stem, leaves and roots were carefully removed from each seedling and dried separately at a constant temperature of 72° C (about 24 hours) in a RIOSSA oven with forced air circulation, model HSF-41 (Metler Toledo, Mexico). Once constant, the weight was determined using a digital scale with an accuracy of 0.01 mg. This process was made for each of the samples.

2.5.9. Total Nitrogen Content

The samples were dried at 72°C for 48h in a RIOSSA oven with forced air circulation, model HSF-41 (Metler Toledo, Mexico). The total nitrogen content was determined using the Kjeldahl digestion technique [20].

2.6. Experimental Design

The experimental design adopted was a randomized design based on Snedecor and Cochran [21], with six replications. The experimental unit consisted of 100 plants and the sampling size was 10 plants randomly selected

2.7. Statistical Analysis

For the statistical analysis, we used one-way analysis of variance (ANOVA), as well as multiple comparisons, using the Tukey HSD procedure for each variable and treatment. The level of statistical significance was set at (p < 0.05) for all the analyses within a confidence interval of 95%. We conducted the statistical analysis using the OriginPro[®] package for Windows version 8.

3. Results and Discussion

3.1. Seedling Height

Greater seedling growth was observed with nitrogen treatment 16 mEq L^{-1} (11.2 ± 2.19 cm) and slower growth, as expected, was control group 0 mEq L^{-1} (4.87 ± 0.88 cm). The seedling height showed that treatments with concentrations of 8 and 12 mEq L⁻¹ were not significantly different (Table 1 (a)). Producing seedlings over 15cm height is not convenient, since the wind can break their stem at the time of transplantation. In addition, seedlings over 15cm demand more water when transplanted and their roots may probably be insufficient for the entire plant in the early days of transplantation. Treatment 16 mEq L^{-1} , therefore, proved to be the most suitable to obtain seedlings with the specified height for transplantation (Fig 2), i.e., below 15cm in less time compared to the other treatments. These results match those reported by Delgado [22], who evaluated nitrogen rates of 0, 4, 8, 12 and 16 mEq L⁻¹ in pepper (Capsicum annuum L.), Puya type, and found that a nitrogen concentration of 16 mEq L^{-1} resulted in seedlings with the best morphological characteristics.



Figure 2. Typical image of tomato crop (Lycopersicon esculentum Mill.) in a commercial greenhouse

3.2. Stem Diameter

The stem diameter indicates the quality of the tomato seedling. A thicker stem represents a lower probability that the plant will bend during transplantation and thus a higher percentage of rooting. The results of this study evidence that the stem diameter gradually increases when the concentration of nitrogen increases. The larger diameter of the stem resulted from treatment 16 mEq L⁻¹: 3.02mm diameter, representing an increase of over 152% compared with the control group (0 mEq L^{-1} : 1.98 mm), Table 2 (a). Three doses of calcium nitrate were evaluated in a study of tomato seedling growth: 100, 200 and 300 mg L⁻¹. It was reported that the dose of 200mg L⁻¹ provided the best stem diameter (3.5 mm), with no significant differences between treatments with concentrations of 200 and 300 mg L⁻¹. The diameter of the stem does not increase when nitrogen content exceeds 200. A concentration of 300 mg L⁻¹ is equivalent to 21 mEq L⁻¹ [23].

Values are expressed as the mean \pm standard deviation (n = 10). The different superscript letters in the same column indicate a significant difference (p < 0.05) among treatments. For each value in the different treatments for each of the variables, the percentage increase compared to the control group is shown in parentheses.

3.4. Fresh Leaf Weight

3.5. Leaf Area

Like the previous variable, the concentration of nitrogen and the fresh leaf weight are positively correlated. The concentration of 16 mEq L^{-1} resulted in a 6.6x increase (1.25mg) vs. the control group (0.19mg).

Nitrogen has a stronger effect on the leaf area when its concentration increases in the seedlings. Thus, the lowest value was obtained from control group 0 mEq L^{-1} (5.3 ± 7.4 cm^{2}), while the biggest leaf area was obtained with treatment 16 mEq L^{-1} (39.6 ± 11.6 cm²). This represents an increase of 7.5x vs. the control group (Table 2 (a)). The increase in leaf area is of great physiological importance for the plant since it represents a bigger photosynthetic active surface. This favors the production of carbohydrates, which combined with water and assimilated minerals directly intervenes in the synthesis of proteins and other organic compounds that produce plants with more biomass (dry weight) [24]. These results are related to those obtained by Utria-Borges [25], who evaluated the growth response of tomato seedlings (Lycopersicon esculentum Mill.) to the application of biosolids to red ferrallitic compacted soil. He compared the response in the leaf area, seedling height, stem diameter, root length and dry weight (total and organs) of tomato seedlings grown on soil treated with 135g kg⁻¹ of biosolids soil with the response of those grown in natural soil and treated with mineral (urea) fertilizer soil at a rate of 150kg N ha⁻¹. The results showed that seedling growth benefitted from the application of biosolids, since a clear increase was observed in the magnitudes of all the variables analyzed, with similar results obtained with treatments involving a mineral fertilizer. Orea-Lara [26] also tested nitrogen concentrations of 0, 2.5, 5, 10 y 15 mol m⁻³, finding a bigger leaf area in chili seedlings treated with 15mol m⁻³ of N-NO₃⁻.

Values are expressed as the mean \pm standard deviation (n = 10). The different superscript letters in the same column indicate a significant difference (p < 0.05) among treatments. For each value in the different treatments for each of the variables, the percentage increase compared to the control group is shown in parentheses.

 Table 2 (a).
 Results of the studied variables

Nitrogen treatment (mEq/L)	Seedling height (cm)	Stem diameter (mm)	Fresh stem weight (mg)	Fresh leaf weight (mg)	Leaf area (cm ²)
0	$4.9 \pm 0.88^{a}(-)$	$1.98 \pm 0.24^{a}(-)$	0.16 ± 0.04^{a}	0.19 ± 0.04^{a}	$5.3 \pm 7.4^{a}(-)$
4	$7.0 \pm 0.94^{b}(142\%)$	2.27 ± 0.34^{b} (115%)	0.32 ± 0.04^{b} (200%)	0.56 ± 0.09^{b} (295%)	$11.7 \pm 2.4^{b}(220\%)$
8	$7.8 \pm 0.86^{\circ} (159\%)$	2.42 ± 0.32^{b} (122%)	$0.43 \pm 0.12^{\circ}$ (269%)	$0.88 \pm 0.19^{\circ}$ (463%)	$21.8 \pm 4.8^{\circ} (411\%)$
12	$8.5 \pm 1.38^{\circ}(173\%)$	$2.66 \pm 0.32^{\circ}$ (134%)	0.57 ± 0.13^{d} (356%)	$0.89 \pm 0.24^{\circ}$ (468%)	$23.1 \pm 4.9^{\circ}(436\%)$
16	11.2 ± 2.19^{d} (228%)	3.02 ± 0.37^{d} (152%)	0.88 ± 0.25^{e} (550%)	1.25 ± 0.37^{d} (658%)	39.6 ± 11.6^{d} (747%)

Table 2 (b). Results of the studied variables

Nitrogen treatment (mEq/L)	Dry stem weight (mg)	Dry leaf weight (mg)	Dry root weight (mg)	Total nitrogen content (mg Kg)
0	$0.02 \pm 0.0^{a}(-)$	$0.02 \pm 0.00^{a}(-)$	$0.02 \pm 0.00^{a}(-)$	$22.40 \pm 1.56^{a}(-)$
4	0.03 ±0.0 ^b (150%)	$0.06 \pm 0.01^{b}(300\%)$	$0.04\pm0.00^{\rm b}(200\%)$	$35.84 \pm 3.02^{b}(160\%)$
8	$0.04 \pm 0.0^{\circ} (200\%)$	$0.08\pm 0.02^{\rm c}(400\%)$	$0.04\pm0.00^{\rm b}(200\%)$	45.11 ± 4.35 ^c (201%)
12	0.04 ±0.0° (200%)	$0.11 \pm 0.02^{d} (550\%)$	$0.04\pm0.00^{\rm b}(200\%)$	$46.15 \pm 2.60^{\circ}(206\%)$
16	$0.07 \pm 0.0^{\rm d} (350\%)$	0.15 ± 0.03^{e} (750%)	$0.04 \pm 0.01^{b} (200\%)$	53.38 ± 2.89^{d} (238%)

3.6. Dry Stem Weight

In general, the weight of the dry stem gradually increases when the concentration of nitrogen is higher (with the exception of treatments with 8 and 12 mEq L⁻¹). The largest value was obtained with treatment 16 mEq L⁻¹ (0.07 ± 0.02 mg), which represents a value of 3.5x vs. the control group 0 mEq L⁻¹ (0.02 ± 0.0 mg), see Table 2 (b).

3.7. Dry Leaf Weight

Like the previous variables, the higher the concentration of nitrogen the higher the dry weight of the leaf, with p < 0.05 in each treatments. An increase of 7.5 times treatment 16 mEq L⁻¹ (0.15 ± 0.03 mg) vs. the value of the control group (0.02 ± 0.0 mg) was observed, see Table 2 (b).

3.8. Dry Root Weight

Regarding the dry root weight, it significantly increased in all the treatments compared to the control group (p <0.05), albeit no significant differences are observed among treatments 4, 8, 12 and 16 mEq L-1, Table 2 (b). These results mirror those reported by Rodriguez [27], who tested two levels of nitrogen fertilization (150 and 350 mg L⁻¹) in tomato seedlings. In this study, he observed that 150 mg L⁻¹ of nitrogen favored the sprout-root ratio in fresh and dry weights, while 350 mg L⁻¹ favored the number of leaves, the diameter of the stem, the sprout fresh weight and the total fresh weight.

We note that unlike the aerial section of the tomato seedling, root growth is limited by the seedbed cavity. In this experiment, a commercial, rectangular-prism seedbed was used with a capacity of 25 ml and a depth of 7 cm. Increasing the volume of the cell height and capacity might have increased root growth, but this should be carefully evaluated. A cavity of 25 ml per seedling is known to be ideal for the development of enough quality roots for an adequate transplant once the seedling has the desired characteristics. Larger cavities would increase the costs and the need for additional space in the seedlings unit and additional substrate, and would result in fewer seedlings. However, the size of the cavities depends on the final use of the seedling.

3.9. Total Nitrogen Content

In Table 2, we can see the positive effect of increasing the concentration of nitrogen: 16 mEq L⁻¹ (53.38 \pm 2.89 mg kg⁻¹) produced the best reaction. When comparing the different treatments (0, 4, 8, 12 and 16 mEq L⁻¹), we can see similar results for plant height, stem diameter, fresh stem weight, fresh leaf weight, leaf area, dry stem weight and dry leaf weight. This confirms the effect of nitrogen on tomato seedlings, consistent with the results reported in other studies [22, 23, 25].

The weight and leaf area must be considered to analyze

plant growth. In general, plant weight is evaluated as the dry weight. The leaf area represents the size of the assimilation system. Indexes are analyzed in order to define growth, for example, the leaf area ratio (LAR) of a plant, which is the assimilated material per unit of material present [28, 29]. There is also a relation between the leaf area and its dry weight. This ratio is useful for relating the plant's total photosynthetic area to the total respiratory material, thereby giving information concerning the plant's available energy balance. Hunt [30] reported that this ratio increases in tandem with the concentration of nitrogen. This finding is consistent with our study, since we observed that the 16m Eq L^{-1} treatment had the highest LAR value: 146.38. The fastest growing seedlings were those that received the higher concentration of nitrogen. Thus, the features that determine the timing for transplant are different in each treatment at different time intervals. Thirty-five days after being treated with 16 mEq L^{-1} , crop seedlings had the best features for transplant. With the other treatments, we would have to wait until the seedlings showed these characteristics for transplant, which represents a longer production cycle. Delgado used chili puya to analyze the same concentration of nitrogen we used in this study, from sowing to fruiting. A concentration of 16 mEq L⁻¹ accelerated flowering and fruit development up to three weeks vs. the control group. Although this concentration of nitrogen increased production by 21%, it did not help improve the quality of fruit. This means that the seedling stage is appropriate for the application of a maximum of 16 mEq L^{-1} de N- NO₃, and nitrogen fertilizer levels above 16 mEq L⁻¹ are unnecessary in the case of pepper and tomato seedlings.

4. Conclusions

Nitrogen influences the organs of tomato seedlings. This study found that increased nitrogen changed in a favorable and proportional fashion the growth characteristics and morphology of the seedlings and shortened their production time. Thirty-five days after sowing, the best seedling features were obtained with the 16 mEq L^{-1} treatment, which favored the development of better morphological characteristics that determine quality. Taking into account the costs of the inputs such as seeds, containers, disinfectants, substrates, agrochemicals, labor and nutrient doses, it is considered that the difference between using nitrogen fertilization to 16 mEq / L represents an increase in the cost of seedling production in the order of 0.22% considering one hectare with a planting density of 3 plants per m². This represents a minimal investment but ensures optimal quality of seedlings for later transplant.

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