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Nitrate/ammonium ratio effect on the growth, yield and foliar anatomy of grafted tomato plants

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Abstract: The vigorous behaviour of a rootstock modifies the growth and yield of a plant variety or hybrid, altering the plant nutritional requirements. The purpose of this work was to study four ratios of NO_3^-/NH_4^+ (100/0, 92/8, 85/15 and 80/20%) over the growth, leaf anatomy and yield of grafted and ungrafted tomato plants. We used a fully randomised experimental block design with factorial arrangement of 2 × 4 (grafted and ungrafted plants and four ratios of NO_3^- /NH_4^+), on eight treatments in total with four replicates each, using Tukey's mean comparison test ($P \leq 0.05$). The rootstock was 'Silex' by Fito Seeds, with the 'El Arrojado' graft (variety) by Gene Seeds. The graft produced an increase in growth, dry biomass production, stomatal density, trichome number, epidermal cell density, fruits per plant, average fruit weight, fruit size and total soluble solids, in comparison with the ungrafted tomato plants. In most of the assessed variables, the grafted tomato plants gave their best response at an 85/15% NO_3^-/NH_4^+ ratio; while the ungrafted plants performed better at a 92/8% NO_3^- /NH_4^+ ratio. The response of the grafted plants to the different NO_3^-/NH_4^+ ratios suggests that grafting induces tolerance to NH_4^+.

Keywords: stomatal; *Solanum lycopersicum*; yield of fruit; trichomes; epidermal cell

Grafting techniques help to increase the yield, improve the crop development, reduce pathogen attacks, increase the fruit quality, and help plants to more efficiently face abiotic stress (Fernández et. al. 2004; Flores et. al. 2010; Al-Harbi et. al. 2017). The benefits reported as advantages in grafted plants are due to the more efficient water and nutrient use of the rootstock vigorous root system; the vigour that the rootstock transfers to the scion and the foliar modification that the leaves undergo under abiotic stress (Martínez et. al. 2010; Savvas et. al. 2010; Gonzalez et. al. 2017).

Due to the reported advantages, grafting is an increasing trend and, therefore, it is necessary to define the proper plant nutrition leading to higher yields and better fruit quality. Among the important plant nutrients, nitrogen (N) is essential to improve the growth, development, and plays an important role in diverse physiological plant functions (Anjum et. al. 2012). Nitrogen is a core component of amino acids, proteins, nucleotides, chlorophyll, chromosomes, genes, ribosomes and enzymes (Anjum et. al. 2012). Ammonium (NH_4^+) and nitrate (NO_3^-) are the two main forms of N that plants can uptake. The available N that forms in the growing medium has an impact on the development, yield and chemical composition of most crops, causing different physiological and morphological plant responses (Marschner 1995; Bugarín et. al. 1998). Ammonium

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can be toxic to most crops, even at low concentrations (Marschner 1995). The toxicity caused by NH_4^+ has shown to be mitigated with the right combination of NO_3^- / NH_4^+, which can also help achieving better growth and yield in many plant species (Marschner 1995; Liu et. al. 2017). However, the optimal ratio of N sources will depend on the concentration of the N supply, the environmental conditions, the crop developmental stage, and above all, the plant species or the genotype (Marschner 1995; Britto, Kranzucker 2002; Hernández et. al. 2015; Liu et. al. 2017).

Despite the vast literature on the effects of NO_3^-/NH_4^+ mixed nutrition in several vegetable crops, there is not enough information indicating that grafting can alleviate the negative effects of ammonium-based nutrition in sensitive crops like the tomato. The aim of this work was to determine the effect of nitrate/ammonium ratios over the growth, yield, fruit quality and foliar anatomy of grafted and ungrafted tomato plants.

MATERIALS AND METHODS

This research was undertaken during the 2016 crop season in a greenhouse at the Universidad Autónoma Agraria Antonio Narro, Horticultural Department in Saltillo, Coahuila, Mexico. The geographical coordinates are 25°21'N latitude, 101°02'W longitude, and 1 762 m above sea level. The mean annual rainfall is 400 mm and has a mean annual temperature of 12–18 °C.

Genetic material planting and grafting. We used the 'Silex' rootstock by Fito Seeds as the plant material, with the 'El Arrojado' variety by Gene Seeds. Both the rootstock and variety were planted in 200-cell polystyrene trays, using acid peat and perlite as the germination medium (80 : 20% v : v). The variety was planted first, followed by the rootstock 4 days later. Grafting was performed 25 days after planting the rootstock. We used spike grafting. Once grafted, the tomato plants were taken to a healing chamber at 23–25 °C and 80–90% relative humidity. The plants were kept in total darkness during the first two days inside the healing chamber, and during the following 6 days, light (16 h) was alternated with darkness (8 hours). Afterwards, the grafted plants were taken to the greenhouse for acclimatisation, where the temperature ranged between 16–28 °C, at a relative humidity of 70–90%.

Field establishment and crop management. Transplanting was undertaken 15 days after grafting. Black polyethylene 10-litre containers were filled with a blend of acid peat and perlite substrate (75 : 25% v : v). The experimental unit included 4 double-stalk plants, 16 plants per treatment in total. The distance between the rows was 1.8 m, with a 0.25 m spacing between the plants. The treatments assessed the grafted and ungrafted plants at four NO_3^- NH_4^+ ratios (100/00, 92/8, 85/15 and 80/20%) (Table 1). The different concentrations of NO_3^- and NH_4^+ were based on the modifications made to Steiner's nutrient solution (Steiner 1961). The original Steiner's solution did not include NH_4^+ as an N source. The modification was the addition of this N form, at different concentrations, reducing the equivalent amount of the total N in the form of NO_3^- (12 meq/L). However, such a modification decreased the relative anion concentration, and it was necessary to increase the SO_4^{2-} concentration in order to have the same number of anions and cations (Table 1). The nutrient solution formulation considered the chemical properties of irrigation water. We adjusted the solutions' pH to 6.0 ± 0.1 with HNO_3^- , H_3PO_4 and H_2SO_4, and we supplied the nutrient solution in percentages, according to the crop's growth stage. We supplied 50% of the nutrient solution four days after transplanting; 75%, twenty days after transplanting and 100% at flowering, keeping this percentage until the end of the experiment. We applied the irrigation water manually, according to the plants' water require-

Table 1. Nutrient solutions assessed in the grafted and ungrafted tomato crops

NO_3^-/NH_4^+ (%)	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	ΣA	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	ΣC
	(Meq/L)								
100/0	12	1	7	20	0	7	4	9	20
92/8	11.04	1	8.92	20.96	0.96	7	4	9	20.96
85/15	10.2	1	10.6	21.8	1.8	7	4	9	21.8
80/20	9.6	1	11.8	22.4	2.4	7	4	9	22.4

ΣA– anion sum; ΣC– cation sum

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ments, always applying enough volume of the nutrient solution to maintain a leaching fraction of 20%.

Plant growth measurements. The experiment finished 120 days after transplanting, assessing four plants per replicate. The test parameters included the distance between the clusters (on both stalks) and the main stalk diameter (at 5 cm from the stalk base). The root systems were rinsed with drinking water to eliminate the substrate excess and the stalks and roots were separated. The root length was measured. The roots and stalks were kiln dried at 70 °C for 72 h until reaching a consistent weight that could be used to determine the dry weight.

Epidermal sampling and stomatal measurements. These parameters were determined 120 days after transplanting; assessing three plants per replicate. The epidermal impressions of a fully mature leaf, oriented towards the East were taken from each plant between 11:00–11:30 am. The technique included the application of a clear varnish on a surface area of 2 cm^2 located at the centre of the leaf underside, between the secondary veins. Five minutes later, we removed the dry varnish layer with clear adhesive tape, and we placed it on a microscope slide. Three pictures of every epidermal impression were taken at random, obtaining nine pictures per replicate, in total. The pictures were taken using a Carl Zeiss microscope with an embedded camera (Pixera Winder Pro) at 10× in order to determine the stomatal density, the stomatal size, the number of trichomes and the epidermal cell density. To find the stomatal density (SD), we counted the stomata present within the picture's surface area (0.3965 mm^2), according to the formula: SD = the number of stomata/picture's surface area, adjusting the results at 1 mm^2. The stomatal length and stomatal width were measured with the Axion Vision Rel. 4.8 software.

Yield and fruit quality measurements. These parameters were determined by assessing the two stalks of the four plants from each replicate. The test parameters included the total yield per plant, which was obtained after harvesting five fruit clusters from each stalk and weighing them in a highly accurate digital scale (Sartorius, TS 1352Q37). We also counted the fruit numbers, determining the average fruit weight after dividing the total fruit weight per plant, by the total number of fruits per plant. The equatorial fruit diameter and the polar fruit diameter were also determined, by measuring with a digital Vernier gauge (by Autotec). In order to determine the total content of the soluble solids, the hydrogen potential and the fruit firmness, we assessed fruits from the first, third and fifth harvested clusters. The total soluble solids content was measured with a refractometer (Atago N-1E), expressing the value in °Brix. The fruit pH was measured with a potentiometer (by Hanna-pHep). The fruit firmness was determined with a penetrometer (FT-327 Fruit Pressure Tester) in a range of 0–13 kg with a 6 mm needle.

Statistical analysis. In this experiment, we used a fully randomised block design with a factorial arrangement (2 × 4), and four replicates per treatment. The resulting data were submitted to an analysis of variance (ANOVA) and the mean comparison was based on Tukey's test ($P \leq 0.05$), using SAS (Statistical Analysis Systems) version 9.1 software.

RESULTS

Growth and biomass. The parameters of distance between the clusters, main stalk diameter, root length, stalk and root dry weights were significantly affected by the grafting. The NO_3^- /NH_4^+ ratio only affected the root length and root dry weight. Likewise, the interaction among these factors had a significant effect on the root length, as well as on the stalk and root weights (Table 2).

The grafted plants had longer roots. At the 85/15% NO_3^-/NH_4^+ ratio, the root length increased more than with the other ratios (Figure 1). The ungrafted plants had shorter roots, and with an increase in the NH_4^+ concentration in the nutrient solution, their root length decreased even more (Figure 1).

The stalk dry weight increased, as the concentration of the NH_4^+ in the nutrient solution feeding the grafted plants increased. A higher dry weight was obtained with an 80/20% NO_3^- /NH_4^+ ratio. The ungrafted plants recorded higher stalk dry weights with a 92/8% NO_3^- /NH_4^+ ratio (Figure 2a). The root dry weight was higher in the grafted plants, obtaining the best response with an 85/15% NO_3^- /NH_4^+ratio. This parameter was lower at an 80/20% ratio. The ungrafted plants did not show any important increase in the root dry weight, and an 80/20% NO_3^- /NH_4^+ ratio led to a lower root dry weight (Figure 2b).

Foliar anatomy. The plant size, stomatal density, epidermal cell density and quantity of leaf trichomes were significantly impacted by the grafting. Contrary to what happened with the stomatal length, the same parameters were significantly impacted by the NO_3^- /NH_4^+ ratios and finally, the interaction

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Table 2. Growth of the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato plants irrigated with different NO_3^-/NH_4^+ ratios in the nutrient solution

Plants	Distance between clusters (cm)	Main stalk diameter (mm)	Root length (cm)	Stalk dry weight (g)	Root dry weight (g)
Grafted	28.33[a]	14.13[a]	53.09[a]	51.62[a]	17.86[a]
Ungrafted	27.86[b]	13.88[b]	43.50[b]	48.97[b]	12.12[b]
ANOVA $P \leq$	***	*	***	*	***
NO_3^-/NH_4^+ (%)					
100/0	27.96[a]	14.07[a]	50.25[a]	48.59[a]	14.59[b]
92/8	28.13[a]	14.13[a]	47.25[ab]	50.37[a]	15.62[ab]
85/15	28.27[a]	13.99[a]	49.91[ab]	51.75[a]	18.00[a]
80/20	28.02[a]	13.82[a]	45.87[b]	50.48[a]	11.75[c]
ANOVA $P \leq$	NS	NS	*	NS	***
Interaction $P \leq$	NS	NS	**	***	*
CV (%)	2.16	3.15	9.36	13.24	17.63

*($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) – significant, > 0.05 – not significant; the means with the same letter in each column are equal, according to Tukey's multiple comparison test with $P \leq 0.05$. ANOVA – analysis of variance; NS – not significant; CV – coefficient of variation

of both factors influenced all the parameters assessed in the leaves (Table 3).

The ungrafted plants had longer stomata, achieving the highest length at the 100/0% and 85/15% NO_3^-/NH_4^+ ratios. In the grafted plants, the stomatal length increased at the 92/8% and 80/20% NO_3^-/NH_4^+ ratios, in comparison with those plants that did not receive NH_4^+ and the plants that were fed at an 85/15% NO_3^-/NH_4^+ ratio (Figure 3a). Overall, the stomata were wider in the ungrafted plants; however, an 85/15% NO_3^-/NH_4^+ ratio produced a much higher increase; while the grafted plants showed a slight increase in the 92/8% and 80/20% NO_3^-/NH_4^+ ratios, in comparison with plants that did not receive NH_4^+ (Figure 3b). The stomatal density increased in the grafted plants, and it was even higher as the NH_4^+ increased, until reaching 15% in the nutrient solution. The ungrafted plants recorded a higher stoma-

Table 3. Stomatal size, stomatal density, epidermal cell density and quantity of the trichomes in the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato leaves, irrigated at different NO_3^-/NH_4^+ ratios, supplied in the nutrient solution

Plants	Stomatal width (μm)	Stomatal length (μm)	Stomatal density (mm^2)	Epidermal cell density (mm^2)	Trichomes quantity (mm^2)
Grafted	26.61[b]	18.96[b]	117.21[a]	641.30[a]	38.03[a]
Ungrafted	30.22[a]	20.92[a]	110.28[b]	566.05[b]	30.86[b]
ANOVA $P \leq$	***	***	**	***	***
NO_3^-/NH_4^+ (%)					
100/0	28.18[a]	18.72[b]	106.87[c]	583.45[b]	31.28[c]
92/8	28.16[a]	20.39[a]	115.67[b]	646.62[a]	43.90[a]
85/15	28.28[a]	20.16[a]	128.68[a]	636.58[a]	35.94[b]
80/20	29.05[a]	20.50[a]	103.76[c]	548.05[b]	26.65[d]
ANOVA $P \leq$	NS	**	***	***	***
Interaction $P \leq$	***	**	***	***	***
CV (%)	7.46	7.17	9.52	9.13	13.57

*$P \leq 0.05$, ** ($P \leq 0.01$) and *** $P \leq 0.001$ – significant, > 0.05 – not significant; the means with the same letter in each column are equal according to Tukey's multiple comparison test at $P \leq 0.05$; ANOVA – analysis of variance; NS – not significant; CV – coefficient of variation

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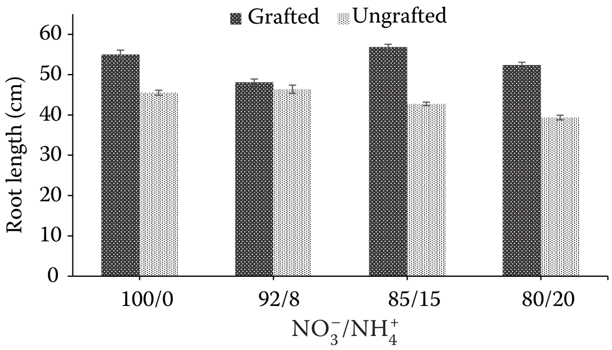

Figure 1. Root length behaviour in the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato plants at different NO_3^-/NH_4^+ ratios supplied in the nutrient solutions

The bars represent the mean standard error

tal density at an 85/15% NO_3^-/NH_4^+ ratio (Figure 3c). The grafted plants had the highest epidermal cell density, peaking at a 92/8% NO_3^- /NH_4^+ ratio; while in the ungrafted plants, this parameter peaked at an 85/15% NO_3^- /NH_4^+ ratio (Figure 3d). The number of trichomes was higher in the grafted plants. Both the grafted and ungrafted plants showed the highest number of trichomes with a 92/8% NO_3^- /NH_4^+ ratio (Figure 4).

Yield. The fruit number, average fruit weight, polar fruit diameter, equatorial fruit diameter and total soluble solids parameters were significantly impacted by the grafting; while the NO_3^-/NH_4^+ ratio affected the fruit number and yield. The interaction between the grafting and the NO_3^-/NH_4^+ ratios only impacted fruit number, average fruit weight and yield (Table 4).

The grafted plants showed lower fruit numbers than the ungrafted plants. However, the latter responded better to an 85/15% NO_3^- /NH_4^+ ratio; while the ungrafted plants showed a better response to a 92/8% NO_3^- /NH_4^+ ratio (Figure 5a). On the other hand, the grafted plants reached higher average fruit weights, when compared to the ungrafted plants. This increase was even higher when the ratio of NH_4^+ in the nutrient solution increased. The best ratio was 85/15% NO_3^- /NH_4^+; however, the ungrafted plants experienced a decrease in the average fruit weight when the NO_3^- /NH_4^+ ratio increased in the nutrient solution (Figure 5b). The ungrafted plants had higher yields when they were fed with a NO_3^- /NH_4^+ ratio at 92/8%; while the grafted plants had higher yields with a NO_3^- /NH_4^+ ratio at 85/15% (Figure 5c).

DISCUSSION

Growth and biomass. The grafting technique has been widely used to improve the crop production, in particular with the *Solanaceae* and *Cucurbitaceae* families, due to its many significant advantages (Huang et. al. 2015). Much research has reported beneficial effects of grafting on the plant development, such as González et. al. (2017) in watermelons and Al-Harbi et. al. (2017) in tomatoes, who obtained higher growth in grafted tomato plants, when compared to ungrafted plants. Our results coincide with the results of their research, since the grafted tomato plants were more vigorous than the ungrafted plants, showing a longer distance between clusters, larger stalk diameter, better root

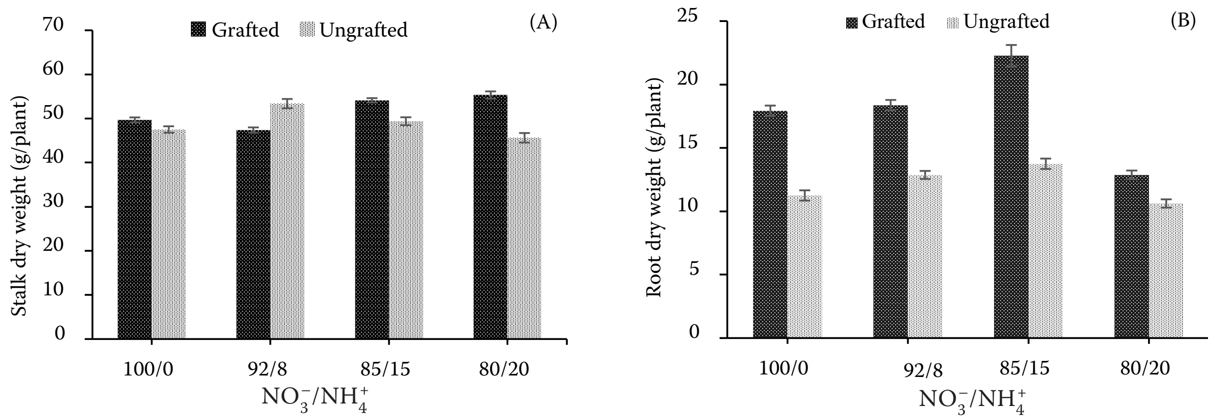

Figure 2. Behaviour of the stalk dry weight (A) and root dry weight (B) of the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato plants at different $NO_3^-NH_4^+$ ratios supplied in the nutrient solution

The bars represent the mean standard error

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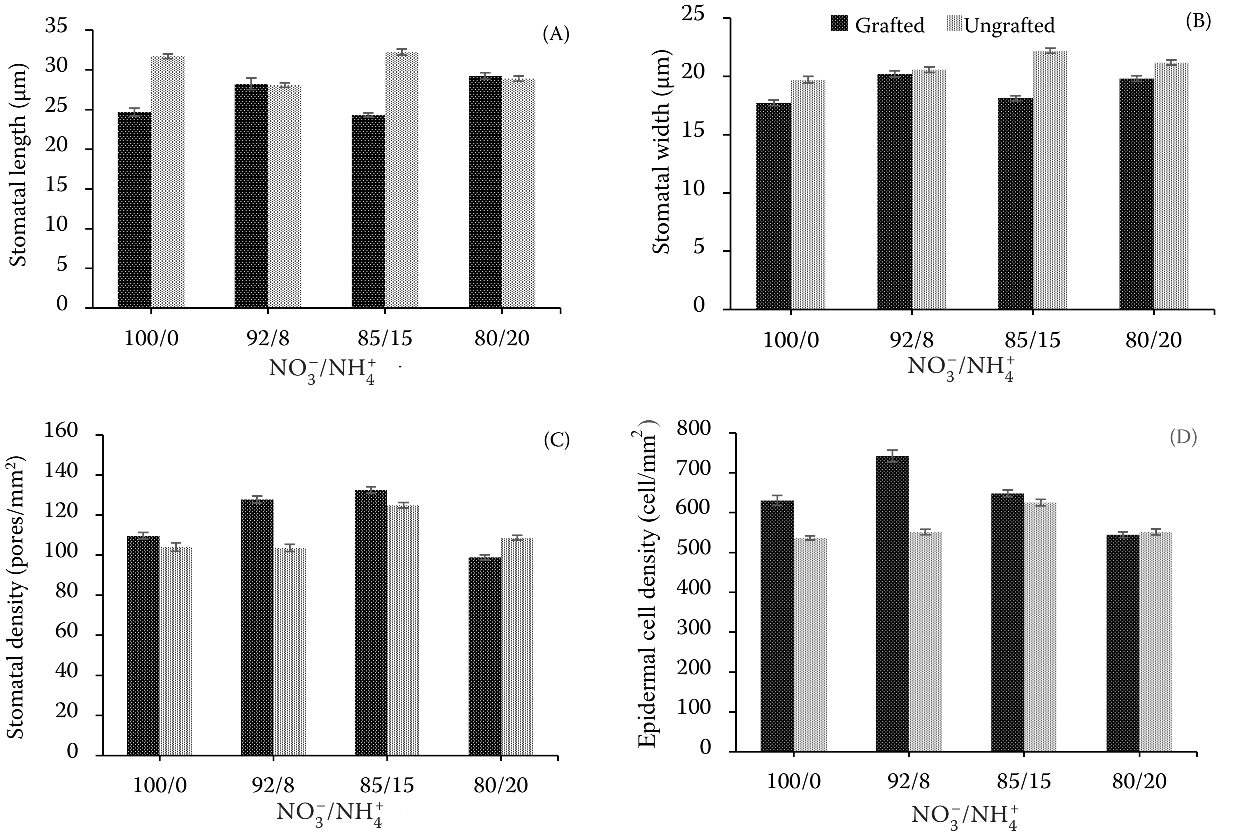

Figure 3. Behaviour of the stomatal length (A), stomatal width (B), stomatal density (C) and epidermal cell density (D) in the grafted ('Silex' + 'El Arrojado 0146') and ungrafted ('El Arrojado') tomato leaves at different NO_3^-/NH_4^+ ratios, supplied in the nutrient solution

The bars represent the mean standard error

length and better stalk and root biomass. In this regard, the effect of grafting on the plant growth and development has been attributed to the physiological interaction among the scion and the rootstock genotypes (Colla et. al. 2010); as well as to the better uptake of nutrients and water, thanks to the vigorous root system of the rootstock (Gonzalez et. al. 2017); the production of endogenous hormones (Zijlstra et. al. 1994) and the enhanced scion vigour (Davis et. al. 2008; González et. al. 2017). Therefore, the joint action of these processes could explain the growth response of the grafted tomato plants ('Silex' + 'El Arrojado') that we assessed over one crop season.

The grafting impact does not only imply stronger resistance against pathogens, but also greater tolerance to abiotic stress sources, such as salinity, heavy metals, nutrients stress, water stress and alkalinity (Rouphael et. al. 2008a, 2008b; Borgognone et. al. 2010; Orsini et. al. 2013; Sánchez et. al. 2013; Mohsenian, Roosta 2015). Ammonium is one the nutrients that can cause plant stress. When this nutrient is present in the plant tissue at toxic levels, it alters the intracellular pH, reduces the respiratory rate, stimulates photorespiration, interferes with the pho-

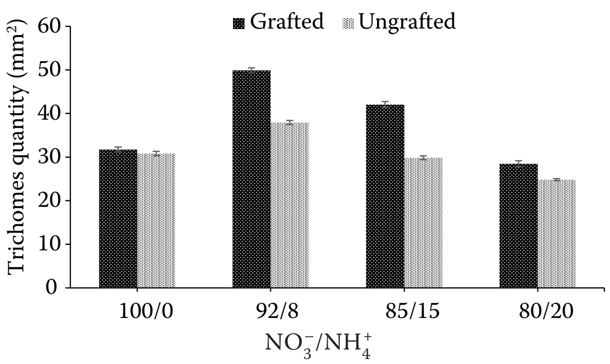

Figure 4. Behaviour of the trichome quantity in the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato leaves at different NO_3^-/NH_4^+ ratios, supplied in the nutrient solution

The bars represent the mean standard error

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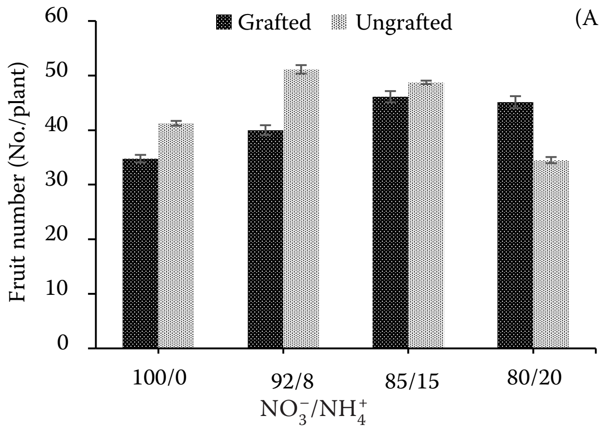
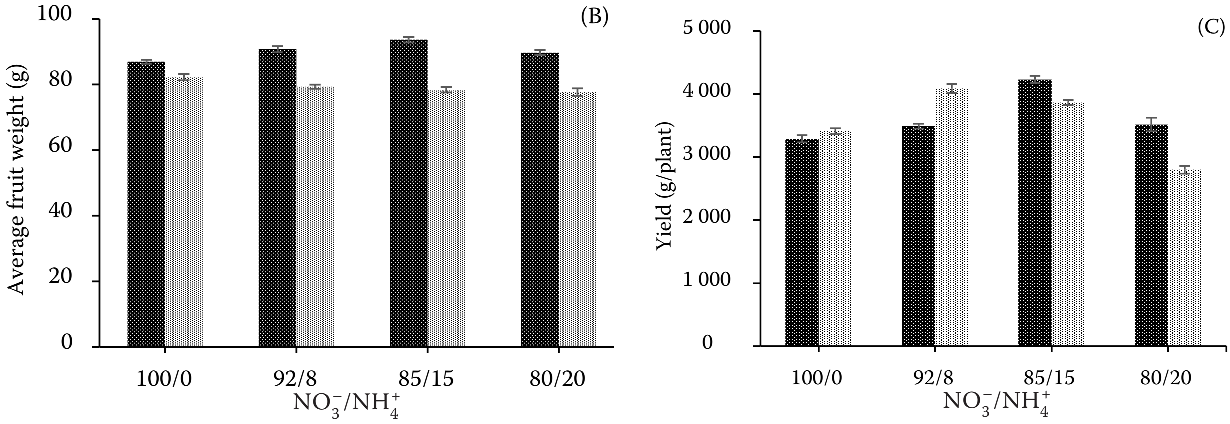


Figure 5. Behaviour of the fruit number (A), average fruit weight (B) and yield (C) of the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato plants at different NO_3^-/NH_4^+ ratios, supplied in the nutrient solution

The bars represent the mean standard error



tosynthesis, increases the oxidising stress and decreases the cationic capacity (Kronzucker et. al. 2001; Britto, Kronzucker 2002; Siddiqi et. al. 2002), leading to lower plant growth. Our research confirmed the resistance of the grafted plants to the stress caused by the addition of NH_4^+. The grafted tomato plants developed better than the ungrafted plants. Therefore, these results show that the 'Silex' + 'El Ar-

Table 4. Components of the yield and fruit quality of the grafted ('Silex' + 'El Arrojado') and ungrafted ('El Arrojado') tomato fruit, irrigated at different NO_3^-/NH_4^+ ratios, supplied in the nutrient solution.

Plants	Fruit number (No./plant)	Average fruit weight (g)	Polar fruit diameter (mm)	Equatorial fruit diameter (mm)	Yield (g/plant)	Total soluble solids (°Brix)	Firmness (kg/cm^2)	pH
Grafted	41.51^b	90.25^a	62.89^a	54.53^a	3633.46^a	3.96^a	8.95^a	4.24^a
Ungrafted	43.91^a	79.41^b	61.21^b	52.92^b	3540.78^a	3.78^b	8.79^a	4.21^a
ANOVA $P \leq$	*	***	**	***	NS	**	NS	NS
NO_3^-/NH_4^+ (%)								
100/0	38.02^b	84.56^a	62.54^a	54.10^a	3350.36^b	3.92^a	8.94^a	4.22^a
92/8	45.56^a	85.02^a	61.80^a	53.83^a	3792.26^a	3.81^a	9.96^a	4.24^a
85/15	47.44^a	86.03^a	62.16^a	53.70^a	4047.50^a	3.88^a	8.69^a	4.21^a
80/20	39.81^b	83.69^a	61.68^a	53.28^a	3158.44^b	3.88^a	8.90^a	4.23^a
ANOVA $P \leq$	***	NS	NS	NS	***	NS	NS	NS
Interaction $P \leq$	***	*	NS	NS	***	NS	NS	NS
CV (%)	10.14	9.39	3.13	3.35	10.59	6.12	5.13	1.51

*($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) – significant, > 0.05 – not significant; the means with the same letter in each column are equal according to Tukey's multiple comparison test at $P \leq 0.05$; ANOVA – analysis of variance; NS – not significant; CV –coefficient of variation

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rojado' graft can help to decrease the adverse effects of NH_4^+. In this regard, Sánchez et. al. (2013) reported that grafted plants are less affected by the NH_4^+ toxicity, because they more efficiently assimilate this cation. The reason is that, under stress conditions, grafted plants increase the activity of the glutamine synthetase (GS) and glutamate synthase (GOGAT) that are responsible for the NH_4^+ assimilation. Furthermore, it has been mentioned that the NH_4^+ toxic effect can be reduced if it is applied in combination with NO_3^-. This combination has promoted growth in many plant species (Marschner 1995; Liu et. al. 2017). This work showed that the grafted tomato plants responded better to an 85/15% NO_3^- /NH_4^+ ratio in terms of the root length and dry weight. The grafted plants also responded better to an 80/20% ratio in terms of the stalk dry weight, while the ungrafted plants could only cope with a 92/8% ratio in terms of the root length and stalk dry weight, and up to an 85/15% ratio, in terms of the root dry weight. These results confirm the higher tolerance of grafted plants to the NH_4^+ toxicity.

Foliar anatomy. Previous research showed that the leaf anatomy of grafted plants undergoes certain changes, due to the vigour transferred by the rootstock to the grafted variety (Ayala et. al. 2010; Camposeco et. al. 2018). Our research work confirmed the effect of grafting on the foliar anatomy. The underside of the tested tomato leaves showed smaller stomata and, therefore, a higher stomatal density, as well as higher numbers of trichomes and epidermal cells, in comparison to the ungrafted plants. Moreover, the smaller stomata and greater stomatal density of the grafted tomato plants can help promote plant growth. According to Hetherington and Woodward (2003), as well as to Orsini et. al. (2011), smaller stomata provide an adaptive advantage to plants, because they can open and close faster and, therefore, they can use water more efficiently in photosynthesis and transpiration. Ayala et. al. (2010) mentioned that the transpiration rate increases as the number of stomata increases, influencing the nutrient uptake and transportation; as well as the physiological efficiency of the leaves to assimilate more CO_2 and transform it into assimilates.

Furthermore, the stomatal frequency and development are affected by the environment (Hetherington, Woodward 2003), as has been reported in tomatoes (Sam et. al. 2000; Salas et. al. 2001), melons (Orsini et. al. 2013) and olives (Bosabalidis, Kofidis 2002) under water and salinity stress. The high concentrations of NH_4^+ in the plant tissue cause toxicity and, in this work, the different leaf anatomic development responses were displayed, according to the different rates of the NH_4^+ in NO_3^- /NH_4^+ ratios; obtaining smaller stomatal sizes at 0 and 15% NH_4^+ in the grafted plants, and greater stomatal density at 15%. Wiesler (1998) reported that the NH_4^+ toxic effect could cause the loss of epidermal turgor, due to a decrease in the concentration of the leaf osmolytes. Thereof, the grafted plants implemented the strategy of developing smaller stomata in larger numbers, in order to use water more efficiently, in particular at an 85/15% NO_3^- /NH_4^+ ratio. This NH_4^+ rate led to better-developed tomato plants, according to Hetherington and Woodward (2003), Ayala et. al. (2010) and Orsini et. al. (2013). Furthermore, in this research work, we saw changes in the number of epidermal cells and trichomes, depending on the rate of NH_4^+ used in the N source ratio, obtaining a better response in the grafted plants with NH_4^+ at 8%. The higher number of epidermal cells and foliar trichomes are plant adaptations to stress conditions, in an effort to control the loss of transpiration water (Bosabalidis, Kofidis 2002; Ayala et. al. 2010). Even when the NH_4^+ (8%) rate resulted in a larger number of epidermal cells and trichomes on the underside of the grafted tomato leaves, the rate of NH_4^+ at 15% promoted the best growth in the grafted plants. These changes represent an adaptative advantage of our grafted tomato plants ('Silex' + 'El Arrojado') under stress conditions, caused by an excess of NH_4^+, in this case.

Yield. Quite often, grafted tomato plants show a significant increase in fruit weight, and, therefore, in the fruit diameter, when compared to ungrafted plants; leading to higher total yields (Passam et. al. 2005; Mascorro et. al. 2012). We only obtained a higher average fruit weight and larger fruit size, but there was no difference in the yield, which might be due to the lower number of fruits in the grafted plants. Contradictory responses in terms of the grafted tomato fruit quality have been reported. Some research works reported the better fruit quality of grafted tomato (Huang et. al. 2015; Rahmatian et. al. 2014), while other works showed that grafted tomato plants did not show any improvement in terms of quality components (Di Gioia et. al. 2010; Al-Harbi et. al. 2017). The results of this work show higher total soluble solids in the grafted plants; while the fruit firmness and pH were not impacted by the type of plant used. Regarding the contradictory reports about changes in the fruit quality of grafted tomato plants, Davis et. al. (2008) indicate that the differences in the results might be partially due to the different production environments, the rootstock/variety combination and the harvesting date. Borgognone et. al. (2013), who worked with grafted and autografted tomato plants at different NO_3^- /NH_4^+ ratios (100/0, 70/30, 30/70 and 0/100%), reported that the yield was not affected by the graft combination, but it was highly influenced by the form of nitrogen, experiencing a linear decrease matching an increase in the NH_4^+ rate. These authors attributed such a decrease to the lower number of fruits. Nevertheless, in this research work, both the type of plant, as well as the NO_3^- /NH_4^+ ratio had an impact on the fruit number, average fruit weight and yield. We were able to supply up to 15% of NH_4^+ to the grafted plants and avoid toxicity, while improving the fruit number, average fruit weight and yield; whereas the highest rate of NH_4^+ that the ungrafted plants withstood was 8%, leading to the best fruit number and the best yield. Furthermore, it was clear that the highest yield of the grafted plants after adding 15% of NH_4^+ was due to the higher average fruit weight and higher fruit number, slightly exceeding the yield reached by the ungrafted plants at the different NO_3^-/NH_4^+ ratios. On the other hand, the higher yield of the ungrafted plants after adding 8% of NH_4^+ was the result of the higher fruit numbers. Likewise, the best yield response of the grafted plants at an 85/15% NO_3^- /NH_4^+ ratio might be due to the better root development and better stomatal density.

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