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Original scientific article

INVESTIGATION ON MORPHOLOGY AND PHYSIOLOGY OF NITROGEN EFFICIENCY IN DIFFERENT PEPPER (*Capsicum annuum* L.) INBRED LINES

Firdes ULAS[1*], Saliha ERDOGDU[1], Halit YETISIR[1], Abdullah ULAS[2]

[1]Department of Horticulture, Faculty of Agriculture, Erciyes University, Kayseri, Turkey
[2]Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Erciyes University, Kayseri, Turkey

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In this study, two hydroponic experiments were carried out in a nutrient solution growth system in a growth chamber to determine the morphological and physiological background of nitrogen efficiency in pepper (*Capsicum annuum* L.). In the first experiment, 16 pepper inbred lines and 2 pepper commercial rootstocks were screened under 2 nitrogen (N) doses (0.3 mM and 3.0 mM N) in a completely randomized block design with 3 replications. In the second experiment, four pepper lines (N-efficient: 21-H-1-1 and AH-2-3, N-inefficient: ERU 1248 and 24-H-6) selected in the first stage of the study were grafted reciprocally and tested under 2 N doses. By using nitrogen efficient lines (21-H-1-1/24-H-6, 21-H-1-1/ERU 1248, AH-2-3/ERU 1248, AH-2-3/24-H-6) as rootstocks increased growth and biomass production compared to non-grafted control plants (N-inefficient), while using N-efficient lines (1248/21-H-1-1, ERU 1248/AH-2-3, 24-H-6/21-H-1-1, 24-H-6/AH-2-3) as scion (ERU caused lower shoot growth than the control (N-efficient). It is also seen that well-developed strong root systems are the most important factor contributing to N use efficiency in pepper.
Key words: NUE-efficiency; inbred line; grafting; rootstock; root morphology

INTRODUCTION

Nitrogen use efficiency (NUE) in plants as yield gain with regards unit of nitrogen (N) present in the soil (HIREL *et al.*, 2007). Also, the explanation from MOLL *et al.* (1982) NUE is in

Corresponding author: Firdes Ulas, Department of Horticulture, Faculty of Agriculture, Erciyes University, Kayseri, Turkey. E-mail: fulas@erciyes.edu.tr, Tel:+90 537 919 3185

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plants as the yield of harvestable product (dry matter)/available soil N (or N supply) (MOLL *et al.*, 1982; SISSON *et al*., 1991). It is the use of available N either resulting from a stronger uptake capacity (uptake efficiency) or more effective use of N taken up for dry matter production use (LAFITTE and EDMEADES, 1994). In a plant, NUE can be increased by genetic modification or plant breeding methods that can have very good nitrogen absorption from the soil and effective utilization (HIREL *et al.,* 2007; GOOD *et al.,* 2004). The nutrient efficiency is associated with the genetic variation among the crop plants and has been well known for at least 92 years (HOFFER, 1926). Up to now, genotypic variations in many crop species have been reported such as rice (FAGERIA and BALIGAR, 2003), maize (MACHADO and FERNANDES, 2001), tobacco (RUIZ *et al.*, 2006), alfalfa (HARRISON *et al.*, 2004; LAWLOR, 2002) and tomato (RUIZ and ROMERO, 1998) and it was observed that developing crop varieties with improved NUE is the identification of key elements that control N assimilation processes (RUIZ *et al.,* 2006). When the plant selection process is completed, the next important issue is the transfer of high NUE traits to other cultivars with lower efficiency but with agriculturally high commercial value (COLLA *et al.*, 2010).

One of the ways to avoid the reduction in crop production which is mainly caused by less NUE in high yielding varieties can be overcome by grafting them onto rootstocks that have the ability to enhance NUE in the scion. In the past, grafting in vegetable crops was used extensively to limit the effects of soil pathogens (LEE, 1994), but the reasons for grafting also the grafted types of vegetables have increased dramatically over the years. Grafting on vegetable plants was first performed in Korea and Japan in the late 1920s by grafting watermelon onto gourd rootstocks to allow for continuous cropping in areas prone to soil-borne diseases (DAVIS *et al.*, 2008). It is also an innovative technique for the suitable cultivation of fruit-bearing vegetables such as tomatoes, bean, eggplant, cucumber, melon in Japan, Korea, the Mediterranean basin, and several European countries (POGONYI *et al.,* 2005). Grafting with vigorous rootstocks can enhance pest and disease resistance, yield, to increase the absorption of nutrients and the mineral content in the aerial portion of the plant, drought and cold tolerance, growth, fruit quality, the tolerance to high and low temperatures and salt tolerance as reported for different crops such as watermelon, melon, tobacco, and tomato (DASGAN *et al.,* 2015; GUNGOR and BALKAYA, 2016; SARABI *et al.,* 2017; ULAS *et al.,* 2019a; b; ULAS *et al.,* 2020).

The use of grafting in pepper plants is not as common as in the rest of horticultural species mainly due to the lack of commercial rootstocks that perform satisfactorily (LEE *et al.*, 2010), in spite of the fact that the use of appropriate rootstocks can be an alternative strategy to avoid or reduce yield losses caused by environmental stresses (SINGH *et al.*, 2017; GIUFFRIDA *et al.*, 2013; PENELLA *et al.*, 2014; 2015; 2016).

So far, slight attention has been paid to the effect of grafting and rootstocks on grafted pepper vegetative growth, fruiting characteristics and yield has been investigated in few studies and with only a limited number of rootstocks (TSABALLA *et al.,* 2013; DONAS-UCLES *et al.,* 2014; SOLTAN *et al.,* 2017; ULAS *et al.,* 2020; AL RUBAYE *et al.,* 2021). As far as we know, few published data are available concerning the N efficiency in grafted vegetable crops (RUIZ and ROMERO, 1999; PULGAR *et al.,* 2000; COLLA *et al.,* 2010; ADAM, 2018; ULAS *et al.,* 2019). ADAM (2018) observed that among four hybrids and 10 local Turkish varieties and four local Ghanaian tomato cultivars two low yielding 'N-inefficient' tomato genotypes (P005 and Karahidir) could be improved when it is grafted onto 'N-efficient' tomato genotypes (Helena F1 and Alt) under low and high N supply conditions. ULAS *et al.* (2019) also stated that a low yielding 'N-inefficient' watermelon cultivar (Crimson Sweet) could be improved when it is grafted onto 'N-efficient' bottle gourd rootstocks (70-07 and 07-45) under low and high N supply conditions. Though, more extensive studies on vegetable crops have to be conducted to better understand whether using vigorous rootstocks could improve NUE under low and high N supply conditions. Therefore, the aim of this study was to determine the genotypic differences in N efficiency of some pepper inbred lines (*Expt. 1*) and further identify rootstock effects on NUE of pepper lines via reciprocal grafting (*Expt. 2*).

MATERIAL AND METHODS

Plant material, treatments and experimental design

Two hydroponic experiments (*Exp. 1* and *Exp. 2*) were conducted by using an aerated hydroponic in a controlled growth chamber situated in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, in Turkey. *In Exp. 1,* 16 pepper (*Capsicum annuum* L.) inbred lines (IL) (21-H-1-1, ERU 457, ERU 1248, 24-H-6, AH-2-3, 33-H-1-1, 17-H-2-3, 29-H-10, 21-H-1-2, ERU 1227, ERU 462, 11B14, B5-11-2, B5-11-4, 5K-3-1, 3SB F1 Sivri) and 2 commercial pepper rootstock (Scarface F1, and Yaocali F1) were tested under low-N (0.3 mM N) and high-N (3.0 mM N) conditions. Regarding to results of *Exp. 1*, four selected pepper IL (N-efficient: 21-H-1-1 and AH-2-3, N-inefficient: ERU 1248 and 24-H-6) were grafted reciprocally in the second experiment. To produce homogenous plantlets for hydroponic growth medium, the seeds were sown in multipots filled with a mixture of peat (pH: 6.0-6.5) and perlite (2v:1v). The seedlings with 2-true-leaves were carefully freed from the peat-perlite growth medium with no root damage and then transferred into 8 L plastic pots filled with a nutrient solution in the growth chamber. At the grafting experiment, when the seedlings developed five or six true leaves, grafting was done by the “tube grafting method” described by ULAS (2019). In both hydroponic experiments, the total vegetation period from transplanting into 8 L plastic pots up to the final harvest was almost 6 weeks. The average day/night temperatures were 25/22°C, the relative humidity was 65-70% and about 350 μmol m^{-2} S^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. Two hydroponic experiments were arranged in a completely randomized block design with three replications and three plants in each pot (replication). In both experiments, all nutrients were supplied to the plants by using an aerated hydroponic applied in 8 L plastic pots. The nutrient solution was prepared by using distilled water containing analytical grade (99% pure) chemicals according to a modified Hoagland and Arnon formulation. The nutrient solution in each 8 L pot was continuously aerated by using an air pump. In both hydroponic experiments, nitrogen was supplied in two different concentrations (Low N: 0.3 mM N, High N: 3.0 mM N) by using two different proportional N sources (75% $Ca(NO_3)_2$ and 25% $(NH_4)_2SO_4$). Furthermore, basic nutrient solution had the following composition (μM): K_2SO_4 (500); KH_2PO_4 (250); $CaSO_4$ (1000); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8); $MnSO_4$ (0.4); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80). All nutrients were replaced when the N concentration of the nutrient solution in the 3.0 mM N rate pots fell below 0.3 mM, as measured daily with nitrate test strips (Merck, Darmstadt, Germany) by using a NitracheckTM reflectometer.

Harvest, shoot and root dry weight measurements

In both hydroponic experiments for the fresh and dry weight determination, grafted and non-grafted plants were harvested by separating them into stems, leaves and roots. Plant materials were dried in a forced-air oven for 72 h at 70°C to determine dry weights. And then they were weighed on an electronic digital scale. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems).

Root morphological measurements

In both hydroponic experiments, the plant root morphological parameters such as root length (cm), root volume (cm^3) and root diameter (mm) of the plants were measured by using a special image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) in combination with Epson Expression 11000XL scanner. From each harvested fresh root samples almost 5.0 g sub-samples were taken. The samples were placed in the scanner's tray. Water was added and with the aid of a plastic forceps, the roots were homogeneously spread across the tray; and the scanning and analysis were done by the WinRhizo system's interface on a computer connected to the scanner. The total plant root length and volume was then determined as the ratio of sampled root fresh weight to the total root fresh weight.

Leaf area and photosynthetic activity measurements

In both hydroponic experiments, the leaf area of the plants was measured destructively during the harvesting process by using a portable leaf-area meter (LI-3100, LI-COR. Inc., Lincoln, NE, USA). The total leaf area was recorded in centimeter square (cm^2). Prior to harvest, non-destructive measurements of the leaf-level CO_2 gas exchange (μmol CO_2 m^{-2} s^{-1}) were done in a controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf net photosynthesis measurement was performed on the youngest fully expanded leaves, using four replicate leaves per treatment in the 3rd and 5th weeks of the vegetation period.

Leaf chlorophyll content (SPAD) and plant height measurements

For each experimental treatment, SPAD readings were taken with the Minolta SPAD-502 chlorophyll meter. During the growth period, two series of SPAD 502 chlorophyll meter readings were performed at the center of the leaves on the fully expanded youngest leaf for each treatment. Plant height (cm) was measured as the distance between the pot surface and the tip of the plant by using a ruler.

Leaf total chlorophyll (a+b) and carotenoid content measurements

A day before harvesting, 100 mg (0.1 g) of fresh leaf samples from each replication of the two treatments were taken for measuring the leaf total chlorophyll and carotenoid contents using UV-VIS Spectroscopy. The samples were put into 15 ml capped containers where 10 ml of ethyl alcohol of 95% concentration was added. They were then kept in darkness at room temperature overnight, to allow for the extraction of the leaf pigments. Measurements were done using the spectrometer (UV/VS T80+ of PG Instruments Limited, UK) at wavelengths of 470 nm, 648.6

nm and 664.2 nm. Total chlorophyll (Total-Chlo) and total carotenoids (TC) were then estimated from the spectrometric readings using the formulae of LICHTENTHALER (1987).

Total-Chlo (mg/g plant sample) = [(5.24 WL664.2- 22.24 WL648.6 x 8.1]/ weight of plant sample (g)

TC (mg/g plant sample) =[(4.785 WL470 + 3.657 WL664.2) -12.76 WL648.6) x 8.1]/ weight of plant sample (g)

Note: WL470, WL648.6 and WL664.2 refer to spectrometric readings at wavelength 470 nm, 648.6 nm, and 664.2 nm respectively.

Shoot nitrate reductase (NR) activity measurement

Nitrate reductase (NR) activity in the shoot was determined following the method proposed by HARLEY (1993). At harvesting fresh plant samples were taken and chopped into pieces; 2 grams of the latter were placed in each of two falcon tubes and labeled time-0 (T0) and time-60 (T60). The tubes were covered with aluminium foil to be screened from light. 10 ml of assay buffer solution [100 mM phosphate buffer, pH 7.5; 30 mM KNO_3; 5%(v/v) propanol] was added to each tube (T0 and T60). The T0 container was immediately placed into boiling water for 5 minutes, removed and allowed to cool to room temperature. While the T60 was kept for 60 minutes at room temperature; after which it was also placed into boiling water for 5 minutes and allowed to cool to room temperature. To detect nitrite in the assay tubes, the optical density (OD) of each standard tube was determined at 540 nm wavelength in the spectrometer.

Shoot nitrogen analysis

After grinding shoot dry materials, almost 200 mg from each dry plant samples were taken to analyze the shoot N concentration (mg N g^{-1} dw) by using the Kjeldahl Nitrogen Determination Method, introduced by KJELDAHL (1883). After the determination of shoot N concentration, the value was multiplied by total shoot dry matter to calculate the total shoot N content (N uptake) of a whole plant (mg N $plant^{-1}$).

Statistical analysis

Statistical analysis of both nutrient solution experiments data was performed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of genotype (*Exp. 1*) or grafting combination (*Exp. 2*) and nitrogen and their interactions on the variables analyzed. Levels of significance are represented by *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Differences between the treatments were analyzed using Duncan's Multiple Test ($P < 0.05$).

RESULTS AND DISCUSSION

Screening Experiment (Exp. 1)

Based on the screening experiment (*Exp. 1*) the results showed that shoot dry matter, shoot N uptake, shoot N concentration, total leaf area, photosynthesis and leaf nitrate reductase activity (NRA) of 18 pepper IL were significantly ($P<0.001$) affected by different rates of N supply (Table 1, Table 2, Fig. 1 and Fig. 2).

Table 1. Plant height, shoot dry matter and root dry matter of pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 1	Plant height (cm/plant)		Shoot dry matter (g/plant)		Root dry matter (g/plant)	
Pepper IL	Low N	High N	Low N	High N	Low N	High N
21-H-1-1	25.5 de	29.6 CD	6.93 a	16.43 AB	4.65 a	2.70 A
ERU 457	29.0 bc	33.5 B	6.47 a	11.00 EF	3.50 b	2.55 AB
ERU 1248	21.5 hi	26.0 EF	4.07 g	11.77 DEF	2.50 e-h	1.76 C-F
24-H-6	19.0 hi	24.0 FG	4.03 g	10.13 F	2.37 fgh	1.67 DEF
AH-2-3	25.5 de	28.0 DEF	6.30 b	17.07 A	2.75 c-f	1.95 CDE
33-H-1-1	21.0 i	23.0 G	3.40 h	7.63 G	1.95 h	1.40 F
17-H-2-3	22.8 gh	25.5 FG	4.40 fg	7.50 G	3.05 b-e	1.45 EF
29-H-10	22.5 hi	27.3 DEF	5.20 de	13.40 CD	3.30 bc	2.15 BC
21-H-1-2	26.0 de	29.6 CD	6.23 b	13.40 CD	2.70 d-g	1.85 C-E
ERU 1227	28.0 b	28.3 DE	5.63 cd	15.27ABC	3.20 bcd	2.00 CD
ERU 462	24.6 ef	31.6 BC	5.60 cd	11.70 DEF	2.20 gh	1.80 C-E
11B14	25.5 de	30.5 CD	5.10 de	10.00 F	1.95 h	1.40 F
B5-11-2	23.6 fg	30.5 CD	4.40 fg	14.43 C	2.70 efg	0.80 G
B5-11-4	28.3 bc	33.0 BC	5.37 de	15.10 BC	2.40 fgh	1.50 DEF
5K-3-1	28.0 bc	32.0 BC	4.95 ef	12.37 DE	2.80 c-f	1.40 F
3SBF1 Siv	32.0 a	37.0 A	6.10 bc	16.60 AB	2.60 efg	1.73 C-F
ScarfaceF1	28.0 bc	39.0 A	5.0 def	16.63 AB	2.35 fgh	1.50 EF
Yaocali F1	28.0 bc	38.3 A	5.30 de	17.07 A	2.80 c-f	1.92 C-F
Pepper IL	***		***		***	
N rate	***		***		***	
Pep.IL x N	***		***		***	

Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at $P < 0.05$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Under high N supply condition, pepper plants indicated usually a higher performance in shoot growth, N uptake, leaf area development, photosynthesis and enzyme activity than pepper plants grown under low N condition. Increasing the N concentration from low to high level in the nutrient solution increased in shoot dry matter by almost 151%, in shoot N uptake by almost 366%, in shoot N concentration by almost 86%, in total leaf area by almost 227%, in photosynthesis by almost 20% and in leaf NR activity by almost 282%. Our results clearly showed that nitrogen has a distinct positive effect on shoot growth and shoot N uptake that might have subsidized to a high leaf area formation and thus a high photosynthetic and enzymatic (NRA) activity of leaves (SCHULTE AUF'M ERLEY *et al.*, 2007; ULAS *et al.*, 2012). Additionally, in our study, it was shown that shoot dry biomass, shoot N uptake, shoot N concentration, total leaf area, photosynthesis and NR activity enhanced when N supply enhanced. Similar results were also observed by (ULAS, 2020). ULAS (2020) stated that during plant growth and development accessibility of N plays a major role in creating and maintaining a photosynthetic active canopy.

Table 2. Nitrogen uptake, nitrogen concentration and total leaf area of pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 1	Nitrogen uptake (mg/plant)		Nitrogen concen. (mg/g dw)		Leaf area (cm^2/plant)	
Pepper IL	Low N	High N	Low N	High N	Low N	High N
21-H-1-1	169.3 a	756.2 A	24.43 bc	46.01 A	1279 cde	4226 EF
ERU 457	135.3 cd	418.3 GH	20.88fgh	38.01 FG	995 i	3633 GH
ERU 1248	74.8 i	488.0 EFG	18.41 i	41.47CDE	1001 i	3438 HI
24-H-6	66.7 I	442.5FGH	16.54 i	43.65ABC	754 j	3026 IJK
AH-2-3	157.7 ab	755.9 A	25.04 b	44.28 AB	1162 efg	4720 CD
33-H-1-1	72.5 I	301.2 I	21.35 fg	39.51 EF	1001 i	2164 M
17-H-2-3	81.7 hi	296.9 I	18.59 i	39.57 EF	1305 cd	2388 LM
29-H-10	100.4 fg	564.9CDE	19.32 hi	42.16 C-E	1024 hi	3420 HI
21-H-1-2	133.4 cd	542.0 DE	21.41 fg	40.44DEF	1212 def	3929 FG
ERU 1227	127.1 de	586.9 CD	22.58def	38.46 F	1051 ghi	2663 KL
ERU 462	126.4 de	409.1 GH	22.58def	34.81 H	865 j	3321 HIJ
11B14	103.9 fg	359.0 HI	20.37 gh	35.82 GH	1140 fgh	2949 JK
B5-11-2	94.7 gh	600.3 CD	21.54 fg	41.58CDE	1177 ef	4416 CDE
B5-11-4	125.4 de	634.3 B-J	23.38bcd	42.00 B-E	1442 b	4356 DE
5K-3-1	149.3 bc	502.4 EF	30.17 a	40.63DEF	1476 b	4820 C
3SB F1 Siv	148.3 bc	712.9 AB	24.33 bc	42.94BCD	1220 def	6180 A
ScarfaceF1	115.9 ef	737.2 A	23.18cde	44.40 AB	1362 bc	4644 CD
Yaocali F1	114.0 ef	684.1 AB	21.53 efg	40.08 EF	1794 a	5345 B
Pepper IL	***		***		***	
N rate	***		***		***	
Pep. IL x N	***		**		***	

Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at $P < 0.05$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

The differences between pepper IL and the growth response to supplied N, i.e. the interaction between N supply and genotypes were also highly significant ($P<0.001$) in shoot dry matter, shoot N uptake, shoot N concentration, total leaf area, photosynthesis and leaf NR activity (Table 1, Table 2, Fig. 1 and Fig. 2). Under low N condition, the variation among pepper IL ranged between 3.40 and 6.93 g/plant in shoot dry matter, 66.77 and 169.37 mg/plant in shoot N uptake, 16.54 and 30.17 mg/g in shoot N concentration, 754.67 and 1794.33 cm^2/plant in total leaf area, 10.15 and 14.00 µmol/m^2/s in photosynthesis and 0.24 and 1.23 µmol/hour/g in NRA activity. Under low N condition, the highest shoot dry matter was recorded in 21-H-1-1, ERU 457 and AH-2-3, though the lowest was determined in 33-H-1-1 and 24-H-6. These results clearly showed that 21-H-1-1 and AH-2-3 can be characterized as 'N-efficient' due to realizing an above-average yield (shoot dry biomass) under low N supply (ULAS *et al.*, 2019). Based on this reference, the lowest shoot dry biomass yielding pepper IL ERU 1248 and 24-H-6 can be characterized as 'N-inefficient'.

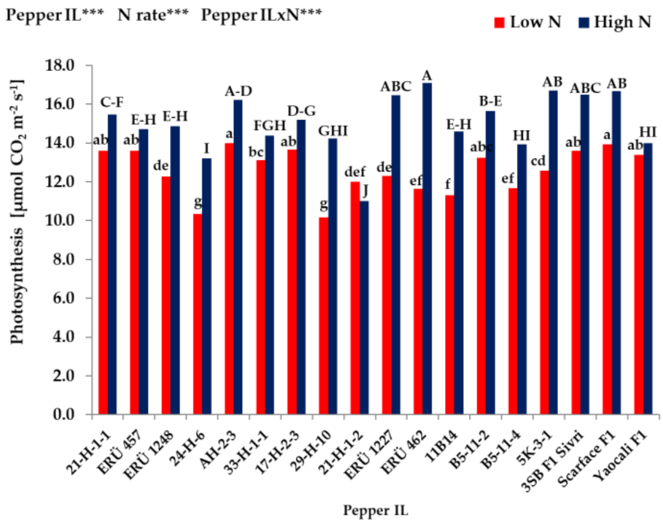

Figure 1. Photosynthesis of the 18 pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply in *Exp. 1*. Bars marked by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between peppers IL within bars on the figure at $P < 0.05$. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

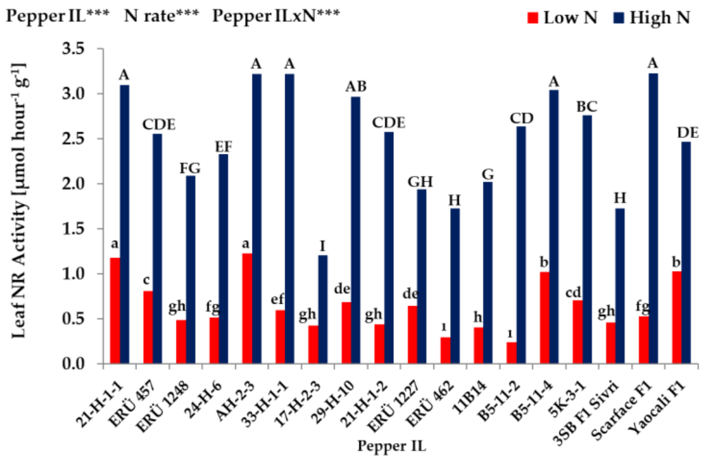

Figure 2. Leaf NR activity of the 18 pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply in *Exp. 1*. Bars marked by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between peppers IL within bars on the figure at $P < 0.05$. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. NR: Nitrate Reductase

Table 3. Leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content of pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 1	Leaf chlorophyll index (SPAD)		Chlorophyll (a+b) content (mg/gr)		Carotenoid content (mg/gr)	
Pepper IL	Low N	High N	Low N	High N	Low N	High N
21-H-1-1	48.43 a	59.30 A	0.81 i	1.97 A	0.26 b	0.43 C
ERU 457	35.23 f	44.10 E	0.90 g	1.50 H	0.16 f	0.53 A
ERU 1248	31.00 g	44.00 E	0.63 l	1.28 L	0.20 cd	0.31 I
24-H-6	29.57 g	45.00 DE	0.63 l	1.52 G	0.15 fg	0.48 B
AH-2-3	44.30 b	52.43 B	1.36 b	1.81 D	0.21 c	0.42 D
33-H-1-1	46.50 ab	52.70 B	0.82 i	1.38 K	0.15 gh	0.26 G
17-H-2-3	40.67 c	46.67 D	1.14 d	1.46 J	0.35 a	0.39 F
29-H-10	41.33 c	50.57 BC	0.38 o	1.81 D	0.13 ij	0.19 N
21-H-1-2	37.47def	49.20 C	0.60 m	1.08 O	0.18 e	0.40 E
ERU 1227	39.13cde	49.27 C	0.89 h	0.83 P	0.14 i	0.31 I
ERU 462	39.87 cd	50.07 C	0.32 p	1.22 M	0.13 ij	0.21 M
11B14	40.30 cd	49.13 C	0.66 j	1.47 I	0.12 jk	0.25 J
B5-11-2	36.63 ef	49.30 C	0.64 k	1.82 C	0.09 l	0.43 C
B5-11-4	38.83cde	49.10 C	1.13 e	1.15 N	0.13 ij	0.35 H
5K-3-1	47.27 a	49.00 C	1.00 f	1.78 F	0.13 ij	0.18 O
3SB F1 Siv	40.30 cd	48.83 C	0.53 n	1.51 GH	0.14 hi	0.22 L
Scarface F1	38.40cde	44.87 DE	1.62 a	1.80 E	0.19 de	0.31 I
Yaocali F1	39.27cde	46.50 D	1.30 c	1.94 B	0.11 k	0.23 K
Pepper IL	***		***		***	
N rate	***		***		***	
Pep. IL x N	***		***		***	

Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at $P < 0.05$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

The results obtained from the *Exp. 1* indicated that plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content of 18 pepper IL were significantly ($P<0.001$) affected by different rates of N supply (Table 1 and Table 3). Pepper plants grown under high N supply produced significantly higher plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content. Increasing the N concentration from low to high level in the nutrient solution increased in plant height by almost 20%, in leaf chlorophyll index (SPAD) by almost 24%, in leaf chlorophyll content by almost 78%, in leaf carotenoid content by almost 99%. Under high N condition, the variation among genotypes ranged between 23.00 and 39.00 cm/plant in plant height, 44.00 and 59.30 SPAD in leaf chlorophyll index, 0.83 and 1.97 mg/g in leaf chlorophyll (a+b) content and 0.18 and 0.53 mg/g in carotenoid content. Though under low N supply, pepper plants displayed a lower performance in plant height, leaf chlorophyll index, leaf chlorophyll (a+b) and carotenoid content as compare to high N supply. The variation among genotypes ranged between 19.00 and 32.00 cm/plant in plant height, 29.57

and 48.43 SPAD in leaf chlorophyll index, 0.32 and 1.62 mg/g in leaf chlorophyll (a+b) content and 0.09 and 0.35 mg/g in carotenoid content. ADAM (2018) also stated that significant genotypic differences in plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content of 4 hybrids and 10 local Turkish varieties and 4 local Ghanaian tomato cultivars was observed under both high and low N supply. Our results verified this study.

Table 4. Total root length, root volume and root diameter of pepper inbreed lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 1	Root length (cm/plant)		Root volume (cm^3/plant)		Root diameter (mm/plant)	
Pepper IL	Low N	High N	Low N	High N	Low N	High N
21-H-1-1	3191 b	2208 A	2931.5 a	2066.5 A	0.36 a	0.35 A
ERU 457	2658 cde	2070 AB	1951.6 def	1680.0CD	0.33 def	0.34 A
ERU 1248	2068 fg	1710 DEF	1802.0 f	1566.0 DE	0.33 def	0.30 EF
24-H-6	2090 fg	1926 C-E	1906.0 ef	1356.5 FG	0.32 efg	0.32 BCD
AH-2-3	3631 a	2177 A	2260.3 bc	2037.6 A	0.35 abc	0.34 A
33-H-1-1	2480 ef	1669 EF	1875.0 ef	1559.5 DE	0.34 cde	0.30 CDE
17-H-2-3	3016 bc	2016 ABC	1968.5 c-f	1680.5CD	0.31 h	0.29 EFG
29-H-10	2529 de	2109 AB	2096.0 c-f	1700.0CD	0.32 fgh	0.29 EFG
21-H-1-2	2298 efg	1776 C-F	2122.5 b-e	1353.5 FG	0.34 cde	0.31 B-E
ERU 1227	1980 g	1378 GH	1936.5 def	1230.5 G	0.33 de	0.32 BC
ERU 462	2483 ef	1830 B-E	2110.5 b-e	1716.5CD	0.34 a-d	0.32 BC
11B14	2579 de	1718 DEF	2048.5 c-f	1699.5CD	0.35 ab	0.30 CDE
B5-11-2	2930 bcd	1878 B-E	2786.0 a	1820.0 BC	0.34 a-d	0.30 CDE
B5-11-4	2358 efg	1218 H	1777.5 g	1278.5 FG	0.35 abc	0.30 CDE
5K-3-1	2585 de	1548 AB	2682.0 a	1441.0 EF	0.29 i	0.28 G
3SB F1 Siv	2442 ef	2066 AB	2759.5 a	1625.6CDE	0.31 gh	0.29 EFG
Scarface F1	2313 efg	2191 A	2220.0bcd	1966.3 AB	0.31 h	0.28 FG
Yaocali F1	2641 cde	1990 A-D	2388.5 b	1696.0 CD	0.34 b-e	0.29 EFG
Pepper IL	***		***		***	
N rate	***		***		***	
Pep. IL x N	***		***		***	

Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at P < 0.05. *P < 0.05, **P < 0.01 and ***P < 0.001.

The results obtained from the *Exp. 1* indicated that the root dry matter, total root length, total root volume and root diameter of 18 pepper IL were significantly (P<0.001) but negatively affected by different rates of N supply (Table 1 and Table 4). Under high N supply, root growth and root morphological development decreased as compare to low N supply. The reason might be under low N supply owing to a higher partitioning of dry matter to the root system (SATTELMACHER *et al.,* 1994). Furthermore, if N supply is not limited, uptake of nitrogen is regulated by the demand of the growing plant (ULAS *et al.*, 2019), however by the amount and efficiency of the root system and also morphological root characteristics such as maximum rooting depth, and root length density at deeper soil layers (SATTELMACHER *et al.,* 1994), if N is limiting. Our results showed that pepper plants also have a physiological behavior similar to previous studies.

The variations among genotypes and the growth response to supplied N, i.e. the interaction between N supply and genotypes, were also highly significant ($P<0.001$) for root dry matter, total root length, total root volume and root diameter (Table 1 and Table 4). Under high N condition, the exhibited variation among IL ranged between 0.80 and 2.70 g/plant in root dry matter, 1218.0 and 2208.3 cm/plant in total root length, 1230.5 and 2066.5 cm^3/plant in total root volume and 0.28 and 0.35 mm/plant in total root diameter. Though under low N supply, the variation among genotypes ranged between 1.95 and 4.65 g/plant in root dry matter, 1980.0 and 3631.3 cm/plant in total root length, 1777.5 and 2931.5 cm^3/plant in total root volume and 0.29 and 0.36 mm/plant in total root diameter. The highest root dry matter, total root volume (also 3 SB F1Sivri, 5K-3-1 and B5-11-2) and total root diameter were shown by the 'N efficient' characterized pepper IL 21-H-1-1, whereas the lowest root dry matter, total root length (also ERU 1227), total root volume (also B5-11-4) and total root diameter (also 5K-3-1) was shown by the 'N-inefficient' characterized pepper IL ERU 1248 and 24-H-6 at low N supply. On the other hand, under high N supply 'N-responsive' characterized pepper IL 21-H-1-1 and AH-2-3 (except root dry matter) displayed higher performance in root growth and morphology than 'N-irresponsive' characterized pepper IL. Based on these results a theory was developed that NUE of a low yielding 'N-inefficient' pepper IL (ERU 1248 and 24-H-6) might be improved when it is grafted onto 'N-efficient' pepper rootstocks (21-H-1-1 and AH-2-3). Therefore, regarding to results of *Exp. 1*, four pepper IL (N-efficient: 21-H-1-1 and AH-2-3, N-inefficient: ERU 1248 and 24-H-6) were grafted reciprocally in the second experiment (see Materials and Methods).

Grafting Experiment (Exp. 2)

Shoot dry matter, shoot N uptake, shoot N concentration, total leaf area, photosynthesis and leaf NR activity of graft combinations were significantly ($P<0.001$) affected by different rates of N supply and also grafting x N supply interaction (Table 5, Table 6, Fig. 3 and Fig. 4). Regardless of graft combinations, increasing the N concentration in the nutrient solution increased the shoot growth, N uptake, leaf area development, photosynthesis and leaf NR activity relative to under low N supply. Increasing N supply, increased the shoot dry matter by almost 118%, the shoot N uptake 338%, the shoot N concentration 99%, the total leaf area 184%, the leaf photosynthesis 125% and the NRA activity 260% at high N condition. Up to now in many studies, it was observed that nitrogen has an evident effect on the growth of the shoot and N uptake of the shoot which might have contributed to a more leaf area formation and therefore a high photosynthetic and enzymatic (NRA) activity of leaves (ORTIZ-MONASTERIO *et al.,* 1997; SCHULTE AUF'M ERLEY *et al.,* 2007; ULAS *et al.,* 2012).

Grafting also had significant positive effects and consequently, the grafted plants showed significantly higher growth performance in shoot growth, N uptake, leaf area development, photosynthesis and leaf NR activity than non-grafted control plants grown at both low and high N rates (Table 5, Table 6, Fig. 3 and Fig. 4). Significant differences were observed between graft combinations and the growth response to supplied N, i.e. the interaction between N supply and graft combination was also highly significant (P<0.001) (Table 5, Table 6, Fig. 3 and Fig. 4).

Table 5. Plant height, shoot dry matter and root dry matter of non-grafted and grafted pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 2	Plant height (cm/plant)		Shoot dry matter (g/plant)		Root dry matter (g/plant)	
Graft Com. (S/R)	Low N	High N	Low N	High N	Low N	High N
21-H-1-1/24-H-6	25.0 bc	29.17 C	5.50 a	12.77 A	2.79 cd	1.89 B
21-H-1-1/ERU1248	24.17 d	28.03 DE	5.46 a	12.97 A	2.74 d	1.96 AB
AH-2-3/24-H-6	24.50 cd	28.67 CD	4.67 b	12.27 A	2.78 cd	1.68 C
AH-2-3/ERU 1248	25.00 bc	31.00 B	5.20 a	12.33 A	2.96 b	1.97 AB
ERU1248/21-H-1-1	22.67 e	26.73 FG	3.53 d	7.30 B	2.43 f	1.69 C
ERU 1248/A-H-2-3	22.00 ef	27.17 EF	3.61 d	7.38 B	2.50 e	1.72 C
24-H-6/21-H-1-1	22.00 ef	26.23 FG	4.03 c	6.97 B	2.18 g	1.26 D
24-H-6/A-H-2-3	21.90 f	24.10 H	3.82 cd	7.00 B	2.20 g	1.42 D
21-H-1-1	25.57 b	30.33 B	5.57 a	12.93 A	2.83 c	2.10 A
ERU 1248	21.33 f	26.00 G	3.50 d	7.27 B	2.48 g	1.70 C
24-H-6	19.00 g	23.00 I	3.80 cd	6.80 B	2.22 f	1.27 D
A-H-2-3	26.67 a	32.17 A	5.50 a	12.30 A	3.10 a	2.00 AB
Grafting	***		***		***	
N rate	***		***		***	
Graft. x N	***		***		***	

S: Scion, R: Rootstock, Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at P < 0.05. *P < 0.05, **P < 0.01 and ***P < 0.001.

Under both high and low N supply, significantly higher shoot dry matter, shoot N uptake, shoot N concentration, total leaf area, photosynthesis and the NR activity was produced when 'N-efficient' pepper IL (21-H-1-1 and AH-2-3) were used as rootstock compared to the non-grafted control plant. All these results clearly confirmed our hypothesis which suggests that plant growth and NUE of a low yielding 'N-inefficient' pepper IL (ERU 1248 and 24-H-6) can be enhanced when it is grafted onto 'N-efficient' inbred lines (21-H-1-1 and AH-2-3). ULAS *et al.* (2019) stated that shoot growth, nitrogen uptake, nitrogen concentration and total leaf area of a low yielding 'N-inefficient' watermelon cultivar (Crimson Sweet) can be improved when it is grafted onto 'N-efficient' bottle gourd rootstocks (70-07 and 07-45) under low and high N supply conditions. ADAM (2018) also observed that among four hybrids and 10 local Turkish varieties and four local Ghanaian tomato cultivars shoot growth, nitrogen uptake, nitrogen concentration and total leaf area of a low yielding 'N-inefficient' tomato genotypes (P005 and Karahidir) can be improved, when it is grafted onto 'N-efficient' tomato genotypes (Helena F1 and Alt) under low and high N supply conditions. Our results are also in line with those of COLLA *et al.* (2010), who observed that melon plants grafted onto *Cucumis melo* and *Cucurbita maxima* Duchesne X *Cucurbita moschata* Duchesne rootstocks efficiently used the N available in the medium.

Table 6. Nitrogen uptake, nitrogen concentration and total leaf area of non-grafted and grafted pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 2	Nitrogen uptake (mg/plant)		Nitrogen concent. (mg/g dw)		Leaf area (cm^2/plant)	
Graft Com. (S/R)	Low N	High N	Low N	High N	Low N	High N
21-H-1-1/24-H-6	118.75 a	549.10AB	21.60 b	43.00 B	781.83bc	2159.33 B
21-H-1-1/ERU1248	110.09 b	531.87AB	20.16 d	41.00 C	771.73bc	2155.33 B
AH-2-3/24-H-6	101.12 c	531.07AB	21.67 b	43.33AB	800.27ab	2328.00AB
AH-2-3/ERU 1248	119.60 a	526.00 B	23.00 a	42.67 B	765.57bc	2224.33AB
ERU1248/21-H-1-1	67.41 ef	300.97 C	19.11 e	41.23 C	554.93 d	1893.67C
ERU 1248/A-H-2-3	74.86 d	276.26CD	20.76 c	37.42 DE	545.07 d	1940.00C
24-H-6/21-H-1-1	74.27 de	252.95 D	18.41 f	36.31 F	728.68bc	1797.00C
24-H-6/A-H-2-3	73.24 de	256.60 D	19.16 e	36.67 EF	715.53bc	1854.00C
21-H-1-1	111.98 b	569.94 A	20.13 d	44.07 A	780.10bc	2145.67B
ERU 1248	64.19 f	271.14CD	18.34 f	37.31 DE	550.00 d	1902.67C
24-H-6	73.24 de	258.57 D	19.27 e	38.01 D	707.93 c	1553.00D
A-H-2-3	123.90 a	543.04AB	22.52 a	44.15 A	866.40 a	2372.67 A
Grafting	***		***		***	
N rate	***		***		***	
Graft. x N	***		***		***	

S: Scion, R: Rootstock, Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at $P < 0.05$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content of graft combinations were significantly ($P<0.001$) affected by different rates of N supply and also grafting x N supply interaction (Table 5 and Table 7). Regardless of graft combinations, pepper plants grown under high N supply produced significantly higher plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content as compare to low N condition. Increasing N supply increased the plant height by almost 19%, leaf chlorophyll index (SPAD) 35%, leaf chlorophyll content 81%, leaf carotenoid content 144% at high N condition. Similar results were reported by ADAM (2018) and ULAS *et al.* (2019). Grafting also had significant positive effects and consequently, the grafted plants showed significantly higher performance in plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content (Table 5 and Table 7). Under both high and low N supply, significantly higher plant height, leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content were observed and these parameters IL can be enhanced when low yielding 'N-inefficient' pepper IL (ERU 1248 and 24-H-6) is grafted onto 'N-efficient' IL (21-H-1-1 and AH-2-3). These results corresponding with the reported by other authors, also the use of grafted plants increase the yield of vegetables, such as bell pepper cultivars (YASUOR *et al.,* 2013; GARCIA-BAÑUELOS *et al.,* 2017) and pepper inbred lines (ULAS *et al.,* 2020).

Table 7. Leaf chlorophyll index (SPAD), leaf chlorophyll (a+b) and carotenoid content of non-grafted and grafted pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 2.	Leaf chlorophyll index (SPAD)		Chlorophyll (a+b) (mg/gr)		Carotenoid (mg/gr)	
Graft Com.(S/R)	Low N	High N	Low N	High N	Low N	High N
21-H-1-1/24-H-6	45.10 b	57.20 B	1.09 ab	1.92 D	0.19 ab	0.38 A
21-H-1-1/ERU1248	44.43 bc	58.30 A	1.06 b	2.01 C	0.16 d	0.35 CD
AH-2-3/24-H-6	42.20 c	53.97 C	1.03 bc	2.15 B	0.16 d	0.40 A
AH-2-3/ERU 1248	43.20 bc	54.10 C	0.98 cd	1.82 E	0.17 cd	0.31 E
ERU1248/21-H-1-1	31.77 def	44.27 H	0.94 de	1.51 GHI	0.12 e	0.29 F
ERU 1248/A-H-2-3	33.13 d	46.13 E	0.92 de	1.45 I	0.09 g	0.28 F
24-H-6/21-H-1-1	30.90 def	47.50 D	0.89 ef	1.58 FG	0.10 fg	0.36BCD
24-H-6/A-H-2-3	30.00 ef	45.33 FG	0.84 f	1.60 F	0.11 ef	0.34 DE
21-H-1-1	47.50 a	59.07 A	1.15 a	2.04 C	0.20 a	0.38ABC
ERU 1248	32.25 de	44.50 GH	0.91 de	1.48 E-I	0.10 fg	0.27 F
24-H-6	29.50 f	46.47 E	0.88 ef	1.54FGH	0.09 g	0.33 DE
A-H-2-3	44.50 bc	54.63 C	1.06 b	2.22 A	0.18 bc	0.39 A
Grafting	***		***		***	
N rate	***		***		***	
Graft. x N	***		***		***	

S: Scion, R: Rootstock, Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between graft combinations within columns at P < 0.05. *P < 0.05, **P < 0.01 and ***P < 0.001.

The root dry matter, total root length, total root volume and total root diameter of graft combinations were significantly (P<0.001) but negatively affected by different rates of N supply (Table 5 and Table 8). Irrespective of graft combinations, increasing the N concentration in the nutrient solution decreased root growth and root morphological development. Increasing N supply, reversely led to a decline in total root dry matter by almost 34%, the total root length by almost 33%, the total root volume by almost 19% and total root diameter by almost 9% at high N conditions. The reason might be as the N concentration decreased in the nutrient solution the dry matter partitioning to the root system increased (SATTELMACHER *et al.,* 1994; MERRILL *et al.,* 2002) and an enhanced carbohydrate sink strength of the roots (MERRILL *et al.,* 2002).

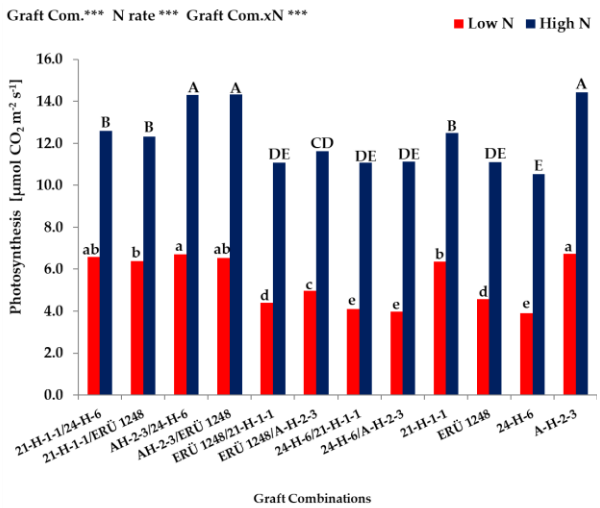

Figure 3. Photosynthesis of non-grafted and grafted pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply in the second experiment Bars marked by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between pepper IL within bars on the figure at P < 0.05. *P < 0.05, **P < 0.01 and ***P < 0.001.

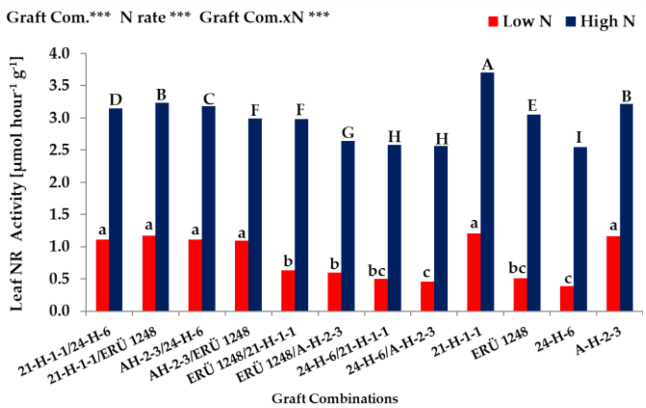

Figure 4. Leaf NR activity of non-grafted and grafted pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply in the second experiment. Bars marked by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between pepper IL within bars on the figure at P < 0.05. *P < 0.05, **P < 0.01 and ***P < 0.001. NR: Nitrate Reductase.

The root dry matter, total root length, total root volume and total root diameter of graft combinations were significantly (P<0.001) but negatively affected by different rates of N supply (Table 5 and Table 8). Irrespective of graft combinations, increasing the N concentration in the nutrient solution decreased root growth and root morphological development. Increasing N supply, reversely led to a decline in total root dry matter by almost 34%, the total root length by almost 33%, the total root volume by almost 19% and total root diameter by almost 9% at high N conditions. The reason might be as the N concentration decreased in the nutrient solution the dry matter partitioning to the root system increased (SATTELMACHER *et al.*, 1994; MERRILL *et al.*, 2002) and an enhanced carbohydrate sink strength of the roots (MERRILL *et al.*, 2002).

Table 8. Total root length, root volume and root diameter of non-grafted and grafted pepper inbred lines grown under low (0.3 mM) and high (3.0 mM) N supply

Exp. 2.	Root length (cm/plant)		Root volume (cm^3/plant)		Root diameter (mm/plant)	
Graft Com. (S/R)	Low N	High N	Low N	High N	Low N	High N
21-H-1-1/24-H-6	3574.6cd	2554.0BC	2834.0 a	2150.3 B	0.36 a	0.34 AB
21-H-1-1/ERU1248	3690.0bc	2625.6 B	2784.3 a	2309.6 A	0.36 a	0.34 A
AH-2-3/24-H-6	3420.3e	2532.0 C	2371.6 b	1999.6 C	0.37 a	0.32 C
AH-2-3/ERU1248	3468.6de	2551.6BC	2456.6 b	1889.3 C	0.37 a	0.33 BC
ERU1248/21-H-1-1	2716.6 h	1814.0 D	1640.3 f	1622.0 D	0.32 b	0.31 D
ERU 1248/A-H-2-3	2891.3 g	1832.3 D	1859.0cd	1413.6EF	0.32 b	0.30 D
24-H-6/21-H-1-1	2822.0 g	1701.0 E	1780.0de	1338.6 F	0.33 b	0.29 E
24-H-6/A-H-2-3	2907.0 g	1603.6 F	1752.0 e	1525.3DE	0.33 b	0.28 E
21-H-1-1	3895.6 a	2775.3 A	2833.6 a	2253.0 A	0.37 a	0.34 A
ERU 1248	2948.0 g	1788.0 D	1930.3 c	1509.0DE	0.32 b	0.30 D
24-H-6	3078.3 f	1679.0EF	1777.0de	1417.3EF	0.33 b	0.29 E
A-H-2-3	3748.6 b	2624.6 B	2471.0 b	2160.3 B	0.375 a	0.33 BC
Grafting	***		***		***	
N rate	***		***		***	
Graft. x N	***		***		***	

S: Scion, R: Rootstock, Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between pepper lines within columns at P < 0.05. *P < 0.05, **P < 0.01 and ***P < 0.001.

Regarding root morphology, significant differences were found among graft combinations and the interaction between N supply and graft combination was also highly significant (P<0.001) (Table 5 and Table 8). Compared to N inefficient non-grafted control plants, N efficient non-grafted plant of 21-H-1-1 and the graft combination 21-H-1-1/ERU 1248 had a higher root dry matter (also N efficient non-grafted plant of AH-2-3 and AH-2-3/ERU 1248), total root length and root volume (also 21-H-1-1/24-H-6 under low N rates) and root diameter (also N efficient non-grafted plant of AH-2-3 and 21-H-1-1, 21-H-1-1/24-H-6, AH-2-3/24-H-6, AH-2-3/ERU 1248 under low N rates) at both low and high N rates. All these results clearly confirmed our starting hypothesis which recommends that plant growth performance of a low yielding 'N-inefficient' pepper IL (ERU 1248 and 24-H-6) can be enhanced when it is grafted onto 'N-efficient' pepper inbred line genotypes (21-H-1-1 and AH-2-3). The grafted N-efficient pepper IL, 21-H-1-1 and AH-2-3, significantly contributed to the growth and biomass production of the N-inefficient pepper plants as compared to non-grafted control plants and thus exhibited a higher rootstock effect for pepper. Our results indicated that the contribution of grafting onto vigorous rootstocks to scion is related to the root power of the rootstocks. Our results were in agreement with the study of COLLA *et al.* (2010) and NAWAZ *et al.* (2017).

Additionally, these results also inveterate that the selection and characterization of pepper inbred line genotypes (N-efficient/N-responsive: 21-H-1-1 and AH-2-3, N-inefficient/N-irresponsive: ERU 1248 and 24-H-6) based on the results of *Exp. 1*, have been obtained appropriately.

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REFERENCES

ADAM, M.B. (2018): Genotypic differences in nitrogen efficiency and rootstock potential of some local Tomato varieties of Ghana and Turkey. Master Thesis (Unpublished). Erciyes University, Graduate School of Natural and Applied Sciences, Kayseri, Turkey, 109p.

COLLA, G., C.M.C. SUAREZ, M. CARDARELLI (2010): Improving nitrogen use efficiency in melon by grafting. HortScience, *45*: 559–565.

DASGAN, H.Y., E. BALACHEVA, H. YETISIR, G, YARSI, O, ALTUNTAS, Y. AKHOUNDNEJAD, A, COBAN (2015): The effectiveness grafting to improve salt tolerance of sensitive melon when the tolerant melon is use as rootstock. Procedia Environmental Sciences, *29*: 268.

DAVIS, A.R., P. PERKINS-VEAZIE, Y. SAKATA, S. LÓPEZ-GALARZA, J.V. MAROTO, S.G.,LEE, R. COHEN (2008): Cucurbit grafting. Critical Rev. Plant Sci., *27(1)*: 50-74.

DONAS-UCLES, F., M. JIMÉNEZ-LUNA, M, DEL, J.A. GÓNGORA-CORRAL, D. PÉREZ-MADRID, D. VERDE-FERNÁNDEZ, F. CAMACHO-FERRE (2014): Influence of three rootstocks on yield and commercial quality of Italian Sweet pepper. Ciência e Agrotecnologia, *38*: 538-545.

FAGERIA, N.K., V.C. BALIGAR (2003): Methodology for evaluation of lowland rice genotypes for nitrogen use efficiency. J. Plant Nutr., *26*: 1315–1335.

GARCIA-BAÑUELOS, M.L., E. SANCHEZ, A.A. GARDEA-BÉJAR, E. MUÑOZ-MARQUEZ, J.M. SOTO-PARRA, D.L. OJEDA-BARRIOS (2017): Nitrogen use efficiency and yield in response graft bell pepper cultivars. Emirates J. Food Agric., *29(6)*: 420-428.

GIUFFRIDA, F., C. CASSANITI, C. LEONARDI (2013): The influence of rootstock on growth and ion concentrations in pepper (*Capsicum annuum* L.) under saline conditions. J. Hortic. Sci. Biotechnol., *88*: 110–116.

GOOD, A.G., A.K. SHRAWAT, D.G. MUENCH (2004): Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends in Plant Sci., *9(12)*: 597-605.

GUNGOR, B., A. BALKAYA (2016): The quantitative effects of local pumpkin rootstock candidates on the vegetative growth of grafted mini watermelon. Bahçe, *2:* 21-26 (in Turkish).

HARLEY, S.M. (1993): Use of a simple colorimetric assay to determine conditions for induction of nitrate reductase in plants. Am. Biol. Teacher, *55*: 161-164.

HARRISON, J., B. HIREL, A.M. LIMAMI (2004): Variation in nitrate uptake and assimilation between two ecotypes of *Lotus japonicus* an their recombinant inbred lines. Physiologia Plantarum, *120(1)*: 124-131.

HIREL, B., J. LE GOUIS, B. NEY, A. GALLAIS (2007): The challenge of improving nitrogen use efficiency in crop plants: towards a more central role or genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot., *58(9)*: 2369-2387.

HOFFER, G.N. (1926): Some differences in the functioning of selfed lines of corn under varying nutritional conditions. Agr. J., *18*: 322-334.

LAFITTE, H.R., G.O. EDMEADES (1994): Improvement for tolerance to low soil-nitrogen in tropical maize. 1. Selection criteria. Field Crops Res., *39*: *1*–14.

LAWLOR, D.W. (2002): Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J. Exp. Bot., *53(370)*: 773-787.

LEE, J.M. (1994): Cultivation of grafted vegetables I: current status, grafting methods and benefits. HortScience, *29*: 235–239.

LEE, J.M., C. KUBOTA, S.J. TSAO, Z. BIE, P. HOYOS ECHEVARRIA, L. MORRA, M. ODA (2010): Current status of vegetable grafting: diffusion, grafting techniques, automation. Sci. Hortic., *127*: 93-105.

LICHTENTHALER, H.K. (1987): Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol., *148*: 350–382.

MACHADO, A.T., M.S. FERNANDES (2001): Participatory maize breeding for low nitrogen tolerance. Euphytica, *122*: 567–573.

MERRILL, S.D., D.L. TANAKA, J.D. HANSON (2002): Root length growth of eight crop species in haplustoll soils. Soil Sci. Soc. America J., *66*: 913-923.

MOLL, R.H., E.J. KAMPRATH, W.A. JACKSON (1982): Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization 1. Agr. J., *74(3)*: 562-564.

NAWAZ, M.A., L. WANG, Y. JIAO, C. CHEN, L. ZHAO, M. MEI, Y. YU, Z. BIE, Y. HUANG (2017): Pumpkin rootstock improves nitrogen use efficiency of watermelon scion by enhancing nutrient uptake, cytokinin content, and expression of nitrate reductase genes. Plant Growth Reg., *82*: 233–246.

ORTIZ-MONASTERIO, J.I., K.D. SAYRE, S. RAJARAM, M. MCMAHON (1997): Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci., *37*: 898–904.

PENELLA, C., S.G. NEBAUER, A.S. BAUTISTA, S. LÓPEZ-GALARZA, A. CALATAYUD (2014): Rootstock alleviates PEG-induced water stress in grafted pepper seedlings: Physiological responses, J. Plant Physiol., *171*: 842–851.

PENELLA, C., S.G. NEBAUER, A. QUI˜NONES, A.S. BAUTISTA, S. LÓPEZ-GALARZA, A. CALATAYUD (2015): Some rootstocks improve pepper tolerance to mild salinity throughionic regulation. Plant Sci., *230*: 12–22.

PENELLA, C., M. LANDI, L. GUIDI, S.G. NEBAUER, E. PELLEGRINI, A.S. BAUTISTA, D. REMORINI, C. NALI, S. LÓPEZ-GALARZA, A. CALATAYUD (2016): Salt-tolerant rootstock increases yield of pepper under salinity through maintenance of photosynthetic performance and sinks strength. J. Plant Physiol., *1* (*193*): 1-11.

POGONYI, A., Z. PÉK, L. HELYES, A. LUGASI (2005): Effect of grafting on the tomato's yield, quality and main fruit components in spring forcing. Acta Alimentaria, *34(4)*: 453-462.

PULGAR, G., G. VILLORA, D.A. MORENO, L. ROMERO (2000): Improving the mineral nutrition in grafted watermelon plants: nitrogen metabolism. Biol. Plant., *43*: 607-609.

RUIZ, J.M., L, ROMERO (1998): Tomato genotype in relation to nitrogen utilization and yield. Journal of agricultural and food chemistry, *46(10)*: 4420-4422.

RUIZ, J.M., L. ROMERO (1999): Nitrogen efficiency and metabolism in grafted melon plants. Sci. Hortic., *81(2)*: 113-123.

RUIZ, J.M., R.M. RIVERO, L.M. CERVILLA, R. CASTELLANO, L. ROMERO (2006): Grafting to improve nitrogen-use efficiency traits in tobacco plants. J. Sci. Food Agr., *86*: 1014–1021.

SATTELMACHER, B., W.J. HORST, H.C. BECKER (1994): Factors that contribute to genetic variation for nutrient efficiency of crop plants. Zeitschrift für Pflanzenernährung und Bodenkunde, *157*: 215-224.

SARABI, B., S. BOLANDNAZAR, N. GHADERI, J. GHASHGHAIE (2017): Genotypic differences in physiological and biochemical responses to salinity stress in melon (*Cucumis melo* L.) plants: Prospects for selection of salt tolerant landraces. Plant Physiol. Bioch., *119*: 294-311.

SCHULTE AUF'M ERLEY, G., K.A. WIJAYA, A.,ULAS, H. BECKER, F. WIESLER, W.J. HORST (2007): Leaf senescence and N uptake parameters as selection traits for nitrogen efficiency of oilseed rape cultivars. Physiologia Plantarum, *130*: 519–531.

SINEBO, W., R. GRETZMACHER, A. EDELBAUER (2004): Genotypic variation for nitrogen use efficiency in Ethiopian barley. Field Crops Res., *85*: 43-60.

SINGH, H., P. KUMAR, S. CHAUDHARI, M. EDELSTEIN (2017): Tomato Grafting: A Global Perspective. HortScience, *52*: 1328–1336.

SOLTAN, M.M., F.A. ELAIDY, J.C. SCHEERENS, M.D, KLEINHENZ (2017): Grafting, Scion and Rootstock Effects on Survival Rate, Vegetative Growth and Fruit Yield of High Tunnel-grown Grafted Pepper (*Capsicum annuum* L.) Plants. Advances in Crop Sci. Techn., *5*: 312.

TSABALLA, A., C. ATHANASIADIS, K. PASENTSIS, I. GANOPOULOS, I. NIANIOU-OBEIDAT, A. TSAFTARIS (2013): A. Molecular studies of inheritable grafting induced changes in pepper (*Capsicum annuum*) fruit shape. Sci. Hortic., *149*: 2-8.

ULAS, A., G., SCHULTE AUF'M ERLEY, M. KAMH, F. WIESLER, W.J. HORST (2012): Root-growth characteristics contributing to genotypic variation in nitrogen efficiency of oilseed rape. J. Plant Nutr. Soil Sci., *175*: 489-498.

ULAS, A., E. DOGANCI, F. ULAS, H. YETISIR (2019): Root-growth characteristics contributing to genotypic variation in nitrogen efficiency of bottle gourd and rootstock potential for watermelon: Plants-Basel, *8*: 77.

ULAS, F. (2019): Effects of rootstocks with vigorous root system on plant growth, seed yield and quality of pepper (*Capsicum annuum* L.) inbred lines. Ph.D Thesis. Erciyes University, Graduate School of Natural and Applied Sciences, Kayseri, Turkey.

ULAS, F., A. AYDIN, A. ULAS, H. YETISIR (2019a): Grafting for sustainable growth performance of melon (*Cucumis melo*) under salt stressed hydroponic condition. Eur. J. Sust. Develop., *81*(1): 201-210.

ULAS, F., A. FRICKE, H. STÜTZEL (2019b): Leaf physiological and root morphological parameters of grafted tomato plants under drought stress conditions. Fresenius Environ. Bulletin, *28* (4A): 3423-3434.

ULAS, A. (2020): Growth, root morphology and leaf physiology of watermelon as affected by various rates and forms of nitrogen in the hydroponic system. Int. J. Agric. Environ. Food Sci., *4(2)*: 134-141.

ULAS, A., A. AYDIN, F. ULAS, H. YETISIR, T.F. MIANO (2020): Cucurbita Rootstocks Improve Salt Tolerance of Melon Scions by Inducing Physiological, Biochemical and Nutritional Responses. Horticulturae, *6*: 66.

ULAS, F., H. YETISIR, A.,ULAS (2020): Effects of grafting on fruit yield and leaf nutrient contents of pepper (*Capsicum annuum* L.) inbred lines. Genetika, *52* (3): 1041-1053.

YASUOR, H., A. BEN-GAL, U. YERMIYAHU, E. BEIT-YANNAI, S. COHEN (2013): Nitrogen management of greenhouse pepper production: Agronomic, nutritional, and environmental implications. Hortic. Sci., *48*(10): 1241-1249.

PROUČAVANJE MORFOLOGIJE I FIZIOLOGIJE EFIKASNOG ISKORIŠĆAVANJA AZOTA KOD PAPRIKE (*Capsicum annuum* L.)

Firdes ULAS[1*], Saliha ERDOGDU[1], Halit YETISIR[1], Abdullah ULAS[2]

[1]Departman za hortikulturu, Poljprivredni fakultet, Ercies Univerzitet, Kajzer, Turska.
[2]Departman za proučavanje zemljišta i ishranu biljaka, Poljprivredni fakultet, Ercies Univerzitet, Kajzer, Turska.

Izvod

U ovom radu su izvedena dva hidroponska eksperimenta u hranljivim rastvorima u komori za rast kako bi se utvrdila morfološka i fiziološka pozadina efikasnosti azota kod paprika (*Capsicum annuum* L.). U prvom eksperimentu, 16 inbred linija paprike i 2 komercijalna uzorka paprike su ispitivani u uslovima primene dve doze azota (N) (0,3 mM i 3,0 mM N) u potpuno randomiziranom blok dizajnu sa tri ponavljanja. U drugom eksperimentu, četiri linije paprike (N-efikasne: 21-H-1-1 i AH-2-3, N-neefikasne: ERU 1248 i 24-H-6) odabrane u prvoj fazi studije, ukrštene su recipročno i testirane u uslovima dve primenjene doze azota. Linije sa efikasnim iskorišćavanjem azota (21-H-1-1/24-H-6, 21-H-1-1/ERU 1248, AH-2-3/ERU 1248, AH-2-3/24-H- 6) imale su povećan rast i proizvodnju biomase u poređenju sa kontrolnim biljkama (N-neefikasnim). Takođe se vidi da je dobro razvijen jak korenov sistem najvažniji faktor koji doprinosi efikasnosti upotrebe N kod paprika.

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