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RESEARCH REPORT

Rootstock characteristics contributing to nitrogen use efficiency and morpho-physiological and biochemical responses of grafted tomato under drought stress conditions

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Abstract
Among the abiotic stresses, it is not just only the nitrogen that is the limiting factor in crop growth and yields, but also due to the shortage and uneven distribution of water resources, drought stress has become a most critical circumstances restricting sustainable crop production. The aim of this study was to determine genotypic differences in the Nitrogen Use Efficiency (NUE) of some local and hybrid tomato (*Lycopersicum esculentum* L.) genotypes and to assess whether grafting with rootstocks could improve the NUE and drought tolerance of susceptible tomato scions. Plants were grown hydroponically under high N (3.0 mM) supply treated with drought stress (10% (w/v) PEG 6000) by using an aerated Deep-Water Culture technique in a fully automated growth chamber of Georg-August University, Agricultural Faculty in Göttingen, Germany. The hydroponic experiment was arranged in a completely randomized block design with three replications for ten weeks between July–September 2022. Two tomato genotypes [Goldene Königin F1 (German hybrid variety) and Iraq 5 (obtained from Iraq Crop Research Institute)] were used as scion and two pre-selected tomato genotypes [Karahidir (N-inefficient Turkish local variety) and ALT (N-efficient Ghanaian local variety obtained from Ghana Research Institute] were used as rootstock materials in this study. Although growing under high N supply, shoot growth, as well as root morphological, leaf physiological and biochemical responses and NUE of both grafted and non-grafted (scions) tomato plants were significantly adverse affected by drought stress. On the other hand, the grafted plants usually exhibited highest growth performance than the non-grafted control plants under drought stress indicating that N-efficient and drought tolerant rootstocks significantly contributed to growth and biomass production and thus NUE of susceptible tomato scions. Among the two graft combinations significant genotypic variation existed in drought stress under high N supply. Previously as 'N-efficient' characterized tomato root stock genotype ALT showed higher drought tolerance in the graft combination of Iraq 5/ALT than the 'N-inefficient' characterized genotype Karahidir (KH) in the graft combination of Goldene Königin (GK)/KH. Two non-grafted susceptible scion genotypes varied significantly under drought stress. The scion genotype Iraq 5 usually showed a higher drought tolerance than the scion genotype GK under high N supply. Both rootstocks (ALT and KH) have high rootstock potential for tomato, and their significant contributions to salt tolerance were closely associated with inducing physiological and biochemical responses of scions under high N supply. These traits could be useful for the selection and breeding of drought-tolerant rootstocks for sustainable high-input agriculture in the future. However, to recommend these tomato rootstocks for using in a low-input agriculture, the experiment should be conducted also under a low N condition. Therefore, a next hydroponic study will be conducted by grafting same tomato scions (Iraq 5 and GK) onto the same rootstocks (ALT and KH) under low N (0.3 mM) supply which will be treated with drought stress in the near future.

Keywords Grafting · N-efficiency · Drought · Tomato · Root morphology · WinRHIZO

1 Introduction

Sustainability in food and agricultural production depends on the efficient use of natural resources. However, recently, under the impact of global climate change, to keep food production at the same level as population growth without

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using up or devastating the natural resources is not an easy task for sustainable agriculture (Ulas et al. 2019a). Since, world population is increasing at a rate of 80 million per year and expected to be around 9.0 billion for the year 2037 and 10.0 billion for 2058 (Zeifman et al. 2023). To meet the rapidly growing food demand of the population, food production must be increased by over 70% from the current level (Byrnes et al. 1998). An increase in food production requires adequate application of fertilizers to replace nutrients removed with harvested crops. Today, mineral fertilizers are still an important resource and an essential input for achieving strong crop growth and yields in both high-input and low-input agricultural systems (Ulas et al. 2024). Nitrogen (N) is the most common and widely used essential fertilizer nutrient for increasing crop yields and plant biomass levels (Liu et al. 2014a). Therefore, to secure yields, growers apply more fertilizer than recommended, and so the global use of chemical N fertilizers in the world increased largely during the past five decades (Byrnes et al. 1998). The utilization of nitrogen fertilizer by farmers has increased by 20%, and by 2030, it is expected to rise to 180 million tons (Adam and Ulas 2023). On the other hand, the available N is often a more limiting factor influencing plant growth than any other nutrient in both high-input and low-input agriculture systems (Grindlay 1997). Usually, in low-input agriculture systems, low amounts of N fertilizers are mostly used by peasant farmers, and thus the fertility of the soil is reducing. On the other hand, due to the overuse of nitrogen fertilizers by high-rate agriculture systems, concerns are rising regarding the environmental pollution of both air and water (Brégard et al. 2000).

Among the abiotic stresses, it is not just only the nitrogen that is the limiting factor in crop growth and yields, but also due to the shortage and uneven distribution of water resources, drought stress has become a most critical circumstances restricting sustainable crop production (Miao et al. 2017). Drought stress as a consequence of insufficient rainfall or deficient soil moisture might induce various biochemical, physiological and genetic responses in plants, which severely restricted crop growth and yield (Seki et al. 2007; Ullah et al. 2018; Vadez et al. 2012). It has been worsened in the last decade by global warming all over the world (Gray and Brady 2016).

As a horticultural crop, tomato (*Lycopersicon esculentum* Mill.) has economic significance in the world due to its intensive and wide cultivation particularly in Europe, America and Asia (Saavedra et al. 2017). Due to easy cultivation, short life cycle with high yield and a high content of valuable minerals like iron and phosphorous, vitamin A, C, fibres and lycopene, the tomato is the most consumed vegetable next after potato and onion (Aksoy 2016). Based on the recorded data the world total tomato production is about 181.0 million tons (Mt) on an area of 5.031 thousand ha^{-1}. Among the main producing countries, China is the first with 62.8 Mt, India is the second with 19.0 Mt, and Türkiye is the third with 12.8 Mt tomato production (FAO 2020).

As compared to other vegetable crops, tomato has a high nitrogen (Hernández et al. 2020; Peet and Welles 2005; Bénard et al. 2009; Bristow et al. 2021) and water requirement (Rao et al. 2000) and thus a controlled supply of both nitrogen and water throughout the growing period is crucial for optimal quality and high yields of this crop (Adam and Ulas 2023). However, tomatoes are highly sensitive to water stress (Nuruddin et al. 2003), particularly in the stages of flowering and fruit enlargement (Hao et al. 2019). On the other hand, a correct management of nitrogen nutrition is crucial for the proper development of this crop and for the sustainability of the environment (Albornoz 2016). While a deficiency of N has been correlated with significant losses in tomato production (Silva et al. 2016), the application of high doses of this essential nutrient can lead to a decrease in the shelf life of fruits, producing physiological alterations and an increasing risk of tissue rupture and senescence (Hewett 2006). To improve NUE and drought tolerance of tomato for a sustainable agriculture, combined N and water management strategies that take into consideration improved fertilizers, soil and crop water management practices are necessary. Among these, a way to improve NUE and hinder yield losses in tomato production caused by drought stress in high-yielding susceptible cultivars (as scions) would be to graft them onto resistant genotypes (as rootstocks) capable of improving NUE and drought tolerance of the scions in both low-input and high-input agriculture systems.

Grafting onto suitable rootstocks is an advanced technique in the horticultural area for both open-field and protected suitable cultivation of some Cucurbitaceae (watermelon, melon, and cucumber) and Solanaceae (tomato, eggplant, and pepper) species in Japan, Korea, China, and some other Asian and European countries (Pogonyi 2005; Lee et al. 2010). Previously, several studies (Rivero et al. 2003; Schwarz et al. 2010; Kumar 2019; Ulas et al. 2019c, d, e; Albaceta et al. 2014; Kumar 2015; Ulas 2022; Nawaz et al. 2017; Ulas et al. 2019a, b; 2021a, b) were carried out to determine the contribution of grafting to several abiotic stress tolerance mechanisms of many plant species. For instance, our previous studies on different plant species clearly indicated that grafting has significant contribution to NUE of watermelon (Ulas et al. 2019a, b) and potato (Ulas et al. 2021c), to salt tolerance of pepper (Al rubaye et al. 2021), melon (Ulas et al. 2020a, b; Ulas et al. 2019a) and peppino (Ulas et al. 2021b), to alkali tolerance of melon (Ulas et al. 2019a, b) and watermelon (Ulas et al. 2021a). However, no comprehensive studies were found in the literature regarding combined abiotic stress effects of nitrogen (N) and drought on growth and yield of tomatoes. Therefore, the aim of this study was to determine genotypic

differences in the nitrogen use efficiency (NUE) of some local and hybrid tomato genotypes and to assess whether grafting could improve the NUE and drought tolerance of susceptible tomato scions through examining the changes induced by the rootstocks in root morphological, shoot agronomical, leaf physio-biochemical responses at high N level (3.0 mM) under drought stress (10% (w/v) PEG 6000) treated hydroponic condition. At the next study these two different tomato genotypes (Iraq 5 and Goldene Königin F1) will be grafted on to two different tomato genotypes (Karahidir: N-inefficient and ALT: N-efficient) and will be tested at low N level (0.3 mM) under drought stress treated hydroponic condition in the near future.

2 Materials and methods

2.1 Plant material, treatments, and experimental design

This study was carried out at the Division Plant Nutrition and Crop Physiology, Department of Crop Sciences, Faculty of Agricultural, Georg-August University in Göttingen, Germany in between July–September 2022. A hydroponic experiment was conducted by using an aerated deep-water culture (DWC) technique in a fully automated growth chamber. For the vegetative growth, the average day and night temperatures were 25/22 °C, while the relative humidity was 60–80%. Plants received approximately 350 µmol m^{-2} S^{-1} photon flux in a photoperiod of 16/8 h of light–dark regimes in the controlled growth chamber. Two tomato genotypes [Goldene Königin F1 (German hybrid variety) and Iraq 5 (obtained from Iraq Crop Research Institute)] were used as scion and two pre-selected tomato genotypes [Karahidir (N-inefficient Turkish local variety) and ALT (N-efficient Ghanaian local variety obtained from Ghana Research Institute] were used as rootstock materials in this study.

Tomato seeds were germinated by wrapping in a germination paper and then keeping into tap water filled beaker on 22.07.2022. They were kept in a dark room till the seeds properly germinated. Scion and rootstock seedlings were grafted by tube grafting method on 02.08.2022, whilst non-grafted scion seedlings were considered to use as control plants (Ulas 2019b). Grafting was done in a fully automated growth chamber under a shaded place to prevent wilting of the grafted plants. Briefly, the shoot tip of rootstock was cut off below cotyledons at an angle of 45 with the same for scion above cotyledons. Grafting position was fixed firmly with a tubular silicone clip. During callus formation, seedlings were healed and acclimatized in a tunnel covered with a double-layered plastic film and shade cloth in climate chamber for five days with relative humidity between 60 and 80% and 25/22 °C day and night temperatures (Ulas et al. 2019a). To inhibit the wilting by extreme transpiration and to increase the healing of grafted plants, the tunnel was closed for three or four days for healing and acclimatization. For the next five or six days, opening and closing of tunnel were done to adapt grafted plants to the environmental conditions outside of the plastic tunnel. Grafted plants remained at healing and acclimatization period totally for ten days. After end of healing and acclimatization, seedlings with eight mature leaves were transferred into 8 L plastic pots filled with modified Hoagland nutrient solution that was aerated by an air pump on 22.08.2022. The research was conducted in a completely randomized block design (CRBD) with three replicated blocks and two plants of every non-grafted cultivar and cultivar by rootstock combination in every block treated. A high level of N concentration (3.0 mM) was supplied proportionally by using 75% $Ca(NO_3)_2$ and 25% $NH_4(SO_4)_2$ as the N source. Hydroponic drought stress (10% (w/v) was applied to plants by using water soluble polyethylene glycol (PEG 6000). The solutions in the pots were changed completely two times through the experiment. The N concentration in the nutrient solution measured daily with nitrate test strips (Merck, Darmstadt, Germany) by using a Nitracheck™ 404 (Serial N 52924) reflectometer. The nutrient solution had the following composition (µM): K_2SO_4 (750); KH_2PO_4 (500); $CaSO_4$ (1000); $MgSO_4$ (650); NaCl (50); H_3BO_3 (10); $MnSO_4$ (0.5); $ZnSO_4$ (0.5); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80).

2.2 Harvest, plant growth and biomass partitioning

The hydroponic experiment was terminated on 26.09.2022. Shoot and root were separated into the leaf, stem, and roots to measure fresh weight. Harvested total plant fresh weight samples were measured with the help of an electronic weighing scale, while the fresh root weight was weighed after drying the roots with tissue paper. Separated fresh root parts were covered with a nylon pocket and kept in a cool room (5 °C) for root length measurement. All the plant tissues were dried in a forced-air oven at 74 °C for 72 h for biomass determination until the stable weight was reached. Then, they were weighed on an electronic digital scale. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems) and was expressed in g $plant^{-1}$. The root: shoot ratio was calculated by dividing the root dry weight by the sum of leaf and stem dry weights. Each fully developed leaf was counted as a leaf number and recorded as LN $plant^{-1}$.

2.3 Leaf physiological measurements

The total leaf area of the plants was measured destructively at harvesting by using a leaf area meter (LI-COR Model 3100, LI-COR. Inc., Lincoln, NE, USA). The total leaf area

was recorded in centimeters square (cm^2). During the growth period, the leaf chlorophyll (CHL), flavonoids (FLAV), and anthocyanin (ANTH) contents were measured non-destructively, as actual estimations of leaf pigment concentrations (Cerovic et al. 2012) by using a leaf-single portable sensor the Dualex™ (Force-A, Orsay, France). Also, the Dualex™ calculates proprietary Nitrogen Balance Index (NBI), that is chlorophyll/flavonoids ratio related to N and carbon allocation (Cerovic et al. 2015). Dualex™ operates with a UV excitation beam at 357 nm, corresponding to maximum absorption for flavonoids; another LED operates in green band for anthocyanin; a red reference beam at 650 nm, corresponding to absorption for chlorophyll; and two other reference bands operate in near-infrared. The measurements were carried out on the youngest fully expanded leaves (3rd–4th leaf from the apex) of whole tomato plants, using 4 replicate leaves per treatment in the third and fifth week of the vegetative period. The measurements were carried out at temperatures of 25/22 °C (average day/night temperatures) and a relative humidity of 60–80%. The supplied photon flux in the growth chamber was almost 350 µmol m^{-2} S^{-1} with a photoperiod of 16/8 h (light/dark). Prior to harvest, non-destructive measurements of leaf-level CO_2 gas exchange (µmol CO_2 m^{-2} s^{-1}) were conducted in controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf net photosynthesis measurement was done on the youngest fully expanded leaves (3rd–4th leaf from the apex), using 4 replicate leaves per treatment in the third and fifth week of the growth period. The CO_2 concentration was 400 µmol L^{-1}, and the photosynthetically active radiation (PAR) was 1000 µmol m^{-2} s^{-1}. All gas exchange measurements were carried out between 09:00 and 12:00 on all plants.

2.4 Mineral analysis measurements

To analyze the macro and micronutrient elements in the plant, 0.5 g of dried plant sample (leaf) was ashed at 400 °C for 4 h in an ashing furnace (Protherm, PC4442T). After ashing process, samples were removed and dissolved in 5 ml of HCl with 20% concentration and then filtered with using Blue ribbon ashless filter paper (S&S Filter Paper Circles, 589[3]). The filtered solution was then diluted to a volume of 50 ml with distilled water and 10 ml taken for Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis. ICP-AES results were converted into parts per million (ppm) and percentages (%) where necessary.

2.5 Shoot and root nitrogen analysis

The plant shoot (leaf) and root dry fractions were grinded and then 200 mg dry samples were transferred to analyze N concentration in the shoot and root by using Kjeldahl Nitrogen Determination Method, introduced by Johan Kjeldahl in 1883 (Labconco 1998). After persistence of shoot and root N concentration, value was multiplied by total shoot and root dry matter to assess total shoot and root N content (N uptake) of a whole plant (mg N $plant^{-1}$).

2.6 Calculation of N efficiency components

Nitrogen efficiency in plants is determined by three different nitrogen efficiency components; It is calculated based on nitrogen uptake (NUP), nitrogen use (NUE) and biological production efficiency (BPE) (Sattelmacher et al. 1994).

After nitrogen analysis (mg/g) was performed on the plant dry matter, the total nitrogen taken by the plant from the environment throughout the vegetation was calculated by multiplying the found value with the total dry weight. Then, N efficiency components were calculated according to the formulas below;

1. *Nitrogen uptake efficiency (NUP)* Total amount of nitrogen in upper part of the plant (mg) / Total amount of nitrogen given to the plant with solution during the trial (mg)
2. *Nitrogen use efficiency (NUE)* Total dry matter in the upper part of the plant (mg) / Total amount of nitrogen given to the plant with solution during the trial (mg)
3. *Biological production efficiency (BPE)* Total dry matter in the upper part of the plant (mg) / Total amount of nitrogen in the upper part of the plant (mg)

2.7 Stress tolerance index (STI) measurement

To find effect of stress on plant growth characteristics, stress tolerance index was calculated with the following formula, taking into account the average values of stress and control applications (M_{stress} and $M_{control}$) in tomato (Guellim et al. 2019):

$$\mathrm{STI}\ (\%) = \left[\left(M_{stress}\right) / M_{control}\right] * 100$$

2.8 Statistical analysis

All measured physiological and morphological parameters were analyzed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA, 2004). A two-factorial analysis of variance was performed to study effects of genotype or grafting combination and drought and their interactions under high N condition. Levels of significance are represented by $*P<0.05$, $**P<0.01$, $***P<0.001$, and ns means not significant. The mean separation of treatments was carried out using Duncan's Multiple Test ($p<0.05$).

3 Results and discussion

3.1 Biomass production and partitioning

The results obtained from the hydroponic experiment indicated that shoot and root dry matter production and root: shoot ratio of non-grafted and grafted tomato cultivars were significantly ($p < 0.001$) affected by graft combinations, drought and graft combinations × drought interaction (Table 1). Irrespective of graft combinations, the plants grown under drought stress generally presented a lower performance in shoot and root biomass production than plants examined under control condition. Drought stress under hydroponic condition lead to decrease in shoot dry matter by up to –61.58%, root dry matter –50.49% and root: shoot ratio by up to 24.47% than plants grown under control condition. Several studies stated that drought stress severely influenced plant growth, development and yield (Grant et al. 1989; Patanè 2011; Khalili et al. 2013). In agreement with our study, comparable results were demonstrated by Wang et al. (2023) who studied the effect of drought stress on maize crop. The study elucidated that the plant height, shoot and root biomass production of maize seedlings significantly declined and thus altered the root: shoot ratio under drought stress (− 0.7 MPa water stress induced by 20% PEG) condition. Another corroborative result was shown by Kayak et al. (2023) who stated that substantial alterations in leaf number, shoot and root fresh and dry biomasses in spinach occurred under 50% water stress condition. The drought stress effects on spinach were also studied by Ors and Suarez (2017) under different irrigation levels, and by Seymen (2021) with the altered time periods under water stress conditions. The results of both studies revealed that the drought stress had enormous adverse impact on the photosynthesis of spinach and thus lead to decline in leaf fresh and dry biomasses. Similar to these studies, water stress negatively influenced fresh and dry head weights and flower number per plant at broccoli (Durak and Yıldırım 2017).

Grafted plants exhibited substantially greater growth performance in shoot growth, and root development than non-grafted plants grown at both control and drought stress conditions (Table 1). Significant variations were observed between graft combinations. Also, the growth response to drought, i.e. interaction between drought and graft combination was highly significant ($p < 0.001$). Grafting enhanced shoot dry weight by –6.6% and 207.86%, root dry weight by 6.38% and 204.69%, root: shoot ratio by 15.75% and –10.33% at control and drought conditions, respectively than control plants. These results clearly indicate that powerful rootstocks contribute greatly to shoot growth than to root growth of tomato plants under drought stress condition. These results highly corroborated with several grafting experiments which was conducted to improve the growth, development and tolerance of scion species (pepper, tomato, cucumber) against to drought stress (Ulas et al. 2019a, b; El-Mogy et al. 2022; Padilla et al. 2023). On the other hand, interestingly the grafted plants under non-stressed condition exhibited growth reduction as compared to non-grafted control plants. This could be the reason of the graft incompatibility between scions and rootstocks under non-stress condition. Another possibility could be non-expression of genes which are only active under stress conditions (Ulas et al. 2020a). Similar result was shown by Al Rubaye et al. (2021) who reported that the rootstocks improved the growth and biomass production of pepper genotypes when they were grafted under salt stress, but contrastingly a substantial decrease in growth and biomass production existed under non-stress condition. Another corroborative result

Table 1 Shoot and root dry weight and root:shoot ratio of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions

Grafted combinations	Shoot dry weight [g plant^{-1}]			Root dry weight [g plant^{-1}]			Root: shoot ratio [g g^{-1}]		
(S/R)	Control	Drought	R%	Control	Drought	R%	Control	Drought	R%
Iraq 5	55.71 a	13.45 C	−75.9	8.45 b	2.18 B	−74.2	0.15 b	0.16 B	7.4
Iraq 5/ALT	50.38 a	40.42 A	−19.8	9.74 a	7.90 A	−18.9	0.19 a	0.20 A	0.87
GK	55.07 a	6.73 D	−87.8	4.92 c	1.20 C	−75.7	0.09 c	0.18 AB	99.6
GK/Karahidir	53.09 a	21.71 B	−59.1	4.49 c	2.38 B	−46.9	0.08 c	0.11 C	29.9
STI (%)	38.42			49.51			124.47		
F-Test									
Graft. Comb	***			***			***		
Drought	***			***			***		
Graft. Comb. × Drought	***			***			***		

Values denoted by different letters (lower and upper case letters for control and drought stress, respectively) are significantly different between genotypes within columns at $p < 0.05$. *ns* non-significant. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. *GK* Goldene Königin F1, *R* reduction, *S* scion, *R* rootstock

was demonstrated also by Ulas et al. (2020a, b) who studied the effects of grafting on fruit yield and leaf nutrient contents of pepper under non-stressed condition. Drought stress enhanced shoot dry weight of Iraq 5 and Iraq 5/ALT plants by –75.85 and –19.76%, root dry weight of Iraq 5 and Iraq 5/ALT plants by –74.20 and –18.85%, respectively than non-stressed plants. STI values were 38.42% at shoot dry weight, 49.51% at root dry weight and 124.47% at root:shoot ratio. Under control conditions, there were no significant differences observed among graft combinations regarding shoot dry weight; though under drought stress condition the grafting combination of Iraq 5/ALT formed considerably greater shoot and root dry biomasses and root:shoot ratio (Table 1). This is in agreements with the finding of Padilla et al. (2023) where he also observed that leaf fresh weight in pepper plants was influenced by drought with substantial alterations for two grafting combinations, with reductions of 28% and 83% in Variety/ NIBER and Variety/ Variety respectively, than control conditions. Root fresh biomass was less influenced by drought without substantial changes, decline was 12% and 8% for Variety/ Variety and Variety / NIBER, respectively and respect to their controls. Parallel outcomes have been stated by Coskun (2023). He found that grafted cucumber plants produced significantly higher leaf, stem and root fresh and dry biomasses than non-grafted and self-grafted plants under drought stress as compared to control condition.

3.2 Total leaf number, total leaf area and photosynthesis

The results of our hydroponic experiment indicated that grafted and non-grafted plants under control conditions generated higher total leaf number, total leaf area and photosynthesis than plants grown under drought stress. Total leaf number was substantially ($p < 0.001$) affected by grafting; though there were no significant differences observed among drought and the interaction of grafting × drought stress. Regardless of graft combinations, weak leaf growth and development of plants were recorded in grafted and non-grafted plants as drought stress was increased. Hence, significant declines were observed in total leaf number (–54.71%), total leaf area (–78.75%) and photosynthesis (–48.37%) of tomato plants under drought applications comparing with control condition, respectively. Under control conditions, total leaf number values ranged between 30 and 56 LN plant^{-1}; however, it was 9–34 LN plant^{-1} under drought stress. Similar results have been also reported by Kayak et al. (2023). Drought reduced leaf number by approximately 22.5% in spinach. Xu and Leskovar (2015) has been found that 50% water stress substantially decline leaf number and leaf area formation in spinach. Total leaf area was substantially ($p < 0.001$) influenced by grafting, drought stress and grafting × drought stress interaction. Under control conditions total leaf area values ranged between 4617.1 and 6188.3 cm^2 plant^{-1} however, it was 246.8–2402.7 cm^2 plant^{-1} under drought stress. Gao et al. (2017) stated that water deficit plants have lower LAI, SPAD, and usually suffer from necrosis. Total leaf area was declined under drought; hence, drought substantially reduced radiation use efficiency of maize (Stone et al. 2001; Earl and Davis 2003). Gonzales-Orenga et al. (2023) found that considerable declines in leaf area formation and leaf fresh and dry biomasses were found stressed plants. Photosynthesis was significantly influenced by grafting ($p < 0.001$) and drought stress ($p < 0.05$); though it was not significantly affected by grafting × drought stress interaction. Under control conditions photosynthesis values ranged between 7.53 and 8.61 µmol m^{-2} sn^{-1}, however, it was 0.24–7.67 µmol m^{-2} sn^{-1} under drought stress. Environmental stress can considerably influence chlorophyll content and activities of key enzymes in photosynthesis (Li et al. 2017). As reported in several studies, drought reduced source and sink relationships that may also interfere with N uptake and photosynthesis of maize (Ghosh et al. 2017; Na et al. 2018). A strong water stress for the duration of vegetative growth period completely prevented growth and leaf area formation and reduce yield in maize (Romano et al. 2011; Ghosh et al. 2017). This impact was improved by studied lowered greenness of leaves that is absolutely associated with less N uptake and a subsequent reduction in leaf area formation and photosynthesis (Urban et al. 2021). Source ability is concluded by photosynthetic products assimilated during maize grain filling phase. Sink strength is described by growing grains' capacity to absorb these assimilates (Bonelli et al. 2016).

Grafting had significantly encouraged consequences and grafted plants displayed considerably greater performance in total leaf number (only under drought stress), total leaf area and photosynthesis (only under drought stress) than non-grafted plants grown at both control and drought stress conditions. The decline in total leaf number was rated among –68.77% and –9.33%. Minimum decline was found at Goldene Königin F1/Karahidir (–68.77%), while maximum decline was found in Iraq 5/ALT (–9.33%). Grafting improved total leaf number by 33.49% and 129.85%, total leaf area by 17.67% and 154.93%, photosynthesis by 8.22% and 188.86% at control and drought, respectively than non-grafted ones. Parallel outcomes have been found by Coskun (2023). He stated that leaf number and leaf area enhanced in some grafting combinations under drought stress conditions, whereas in some grafting combinations they were similar to non-grafted cucumber plants. Regarding total leaf area, the reduction values were ranked between –94.65% and –61.17%. Minimum decline was found at non-grafted control plant of Goldene Königin F1 (–94.65%), while the maximum

reduction was observed in Iraq 5/ALT (–61.17%). Previous studies have shown that grafting improved leaf area under drought stress at cucumber plants (Liu et al. 2016; Coskun 2023). Under control conditions the graft combination of Goldene Königin F1/Karahıdır produced higher total leaf number (56 LN plant^{-1}) and photosynthesis (8.61 µmol m^{-2} sn^{-1}) as compared to non-grafted plants, though it was not significant. Under drought stress condition the grafting combination of Iraq 5/ALT produced significantly higher total leaf number (34 LN plant^{-1}), total leaf area (2402.7 cm^2 plant^{-1}) and photosynthesis (7.67 µmol m^{-2} sn^{-1}). In some earlier experiments, it was found that photosynthetic parameters were adversely influenced by drought (Dugasa et al. 2019; Shin et al. 2021; Metwaly et al. 2022; Karimi et al. 2022; Asayesh et al. 2023; Zahedi et al. 2023). Likewise, in our experiment, photosynthesis was reduced considerably in non-grafted ones under drought. Vegetable grafting can avoid decline of photosynthesis under abiotic stresses. In some experiments, it was stated that grafting enhanced chlorophyll content and photosynthetic system in some plants under drought (Penella et al. 2014; Liu et al. 2014b; Shehata et al. 2022). Reduction in photosynthesis was rated among –96.82% and –0.71%. Minimum decline was found at non-grafted Goldene Königin F1 (–96.82%), as maximum decline was found in Iraq 5/ALT (–0.71%). Whole grafted plants had a positive influence on photosynthetic parameters, just differences were established according to variety of the rootstock. STI values were 45.29% at total leaf number, 21.25% at total leaf area and 51.63% at photosynthesis (Table 2). Coskun (2023) stated that grafted cucumber plants produced significantly higher photosynthesis than non-grafted and self-grafted plants under drought stress as compared to control condition.

3.3 Leaf pigments

The relative pigment content in leaves in grafting combinations and tomato plants as a function of drought stress were shown in Fig. 1. In general, NBI (Nitrogen Balanced Index), the total chlorophyll content, (CHL), flavonoid content (FLAV), and anthocyanins (ANTH) contents were negatively affected by increasing the drought stress of the nutrient solutions at the grafted and non-grafted tomato plants. CHL content (Dualex units), NBI (Dualex units) and ANTH (Dualex units) were significantly ($p < 0.001$) affected by grafting, drought stress and grafting × drought stress interaction. Although, FLAV content (Dualex units) was not significantly affected by grafting, and grafting × drought stress interaction; it was significantly ($p < 0.05$) affected by drought stress. Despite the graft combinations, NBI, CHL content, FLAV content, and ANTH content were relatively lower under drought stress conditions. Increasing drought stress at the hydroponic condition decreased CHL content by up to –5.67%, FLAV content by up to 10.91%, ANTH content by up to 7.26%, and NBI by up to –13.05% than plants grown under control condition. Up to now in many studies, it has been observed that drought hinders plant growth, declines chlorophyll content and photosynthetic efficiency, and interrupts metabolism, ultimately affecting losses in crop productivity (Anjum et al. 2017; Ali et al. 2018). Gao et al. (2012) found that chlorophyll content reduced under drought in tomato. Sivakumar and Srividhya (2016) reviewed that chlorophyll content reduced more in drought-sensitive plants than in drought-tolerant plants in tomato.

Generally grafting had convinced influences and thus, grafted plants produced considerably greater chlorophyll and polyphenols contents than non-grafted plants grown at both control and drought stress conditions. Regarding NBI under control condition the graft combination of Goldene Königin

Table 2 Total leaf number, total leaf area, and photosynthesis of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions

Grafted combinations	Leaf number [LN plant^{-1}]			Leaf area [cm^2 plant^{-1}]			Photosynthesis [µmol CO_2 m^{-2} s^{-1}]		
(S/ R)	Control	Drought	R%	Control	Drought	R%	Control	Drought	R%
Iraq 5	30 a	13 B	−55.6	5643 c	1090 B	−80.7	7.53 a	3.93 AB	−47.8
Iraq 5/ALT	38 a	34 A	−9.3	6188 a	2403 A	−61.2	7.72 a	7.67 A	−0.71
GK	40 a	9 B	−77.3	4617 d	247 D	−94.7	7.56 a	0.24 B	−96.8
GK/Karahıdır	56 a	17 B	−68.8	5884 b	1006 C	−82.9	8.61 a	4.38 AB	−49.1
STI (%)	45.29			21.25			51.63		
F-Test									
Graft.Comb	***			***			***		
Drought	n.s			***			*		
Graft.Comb. × Drought	n.s			***			n.s		

Values denoted by different letters (lower and upper case letters for control and drought stress, respectively) are significantly different between genotypes within columns at $p < 0.05$. *ns* non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. *GK* Goldene Königin F1, *R* reduction, *S* scion, *R* rootstock

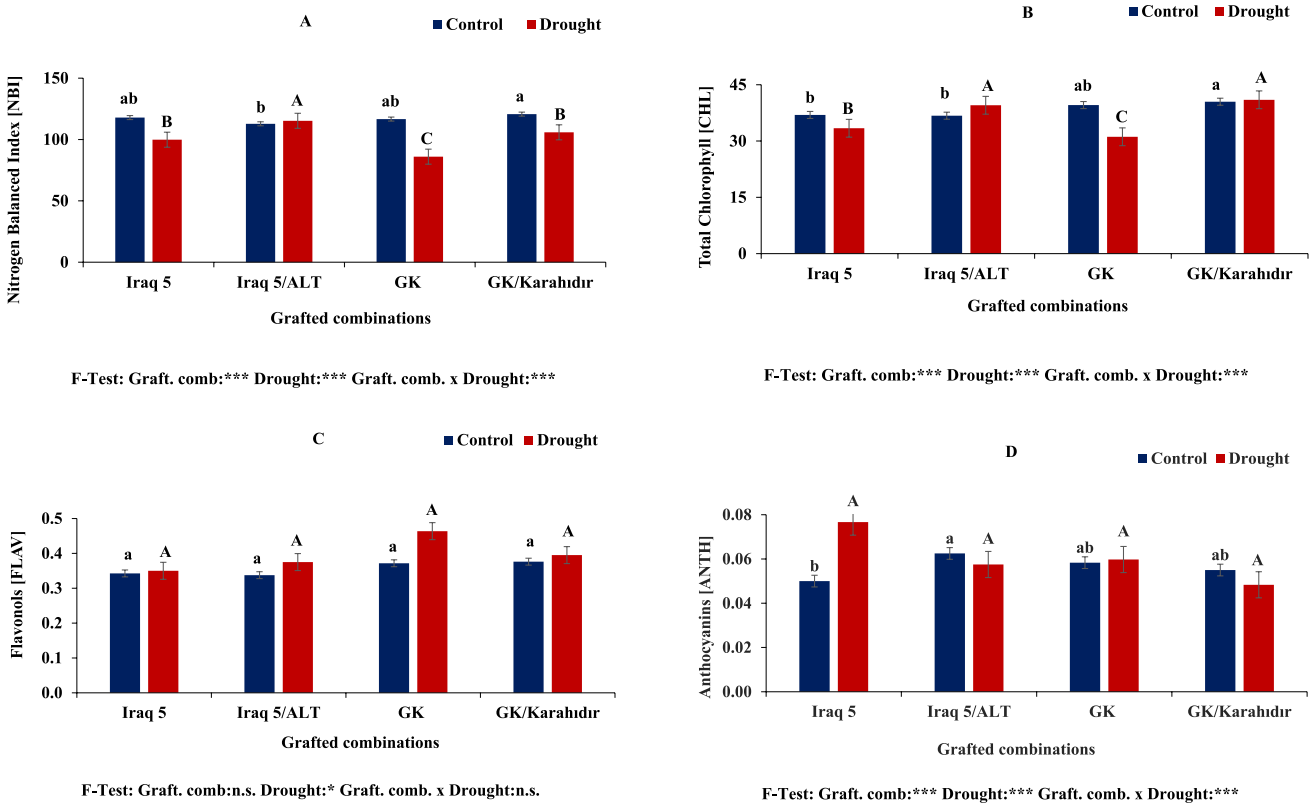


Fig. 1 The nitrogen balanced index(a), chlorophyll(b) and polyphenols (flavonoid(c) and anthocyanins(d)) contents of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions. [1] Values denoted by different letters (lower and upper case letters for control and drought stress, respectively) are significantly different between genotypes within columns at $p < 0.05$. *ns* non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. *GK* Goldene Königin F1

F1/Karahıdır produced significantly higher (120.7) Nitrogen Balanced Index as compared to non-grafted plants. Under drought stress condition the graft combination of Iraq 5/ALT supplied significantly higher (115.27) Nitrogen Balanced Index as compared to non-grafted plants. It was followed by Goldene Königin F1/Karahıdır (105.87), Iraq 5 (99.85) and Goldene Königin F1 (86.03). The reduction in NBI was ranked between –26.26 and 2.16%. The minimum reduction was observed at the graft combination of Goldene Königin F1/Karahıdır (–26.26%), while the maximum reduction was observed at the non-grafted control plant of Iraq 5 (2.16%) (Fig. 1a). In terms of CHL content, the graft combination of Goldene Königin F1/Karahıdır produced significantly higher chlorophyll as compared to non-grafted plants under both control (40.48) and drought stress (40.98) conditions. The reduction in CHL content was rated among –21.35 and 7.55%. Minimum decline was found at Goldene Königin F1/Karahıdır (–21.35%), as maximum decline was found at Iraq 5 (7.55%) (Fig. 1b).

Chlorophyll and nitrogen balance index are absolutely correlated with plant nutritional level (Cartelat et al. 2005; Overbeck et al. 2018). Study with Dualex has stated that chlorophyll index is a good indicator of nitrogen status in grapevine (*Vitis vinifera* L.) (Cerovic et al. 2015), showing a high correlation with the SPAD index (Cerovic et al. 2012). A higher chlorophyll index value has corresponded with bigger fruit production in different cherry, probably because of better nitrogen level and photosynthetic capacity of trees (Overbeck et al. 2018). Regarding FLAV content, among grafted and non-grafted plants there were no significant variations observed under control and drought. Under control condition graft combination of Goldene Königin F1/Karahıdır produced higher (0.38) flavonols; on the other hand, the non-grafted plant of Iraq 5 (0.34) and graft combination of Iraq 5/ ALT (0.34) produced lower flavonols. Under drought stress, the non-grafted plant of Goldene Königin F1 produced higher (0.46) flavonols; on the other hand, the non-grafted plant of Iraq 5 (0.35) produced lower flavonols (Fig. 1c). Secondary metabolites construction, notably phenylpropanoids like flavonoids and flavonols, is induced by various biotic and abiotic environmental stresses (Dixon and Paiva 1995). Comparable outcomes were found by Barthod et al. (2007).

Flavonoid index has shown a high correlation with leaf phenol content (Barthod et al. 2007). Furthermore, in canopies with a high exposure to UV radiation, this index

is greater, while in less illuminated canopies, it tends to decline. Hence, differences in this indicator can reference differences in synthesis of photoprotective compounds in leaves in face of light alterations generated by rain cover (Overbeck et al. 2018). In terms of ANTH content, under control condition the grafted plant of Iraq 5/ ALT (0.063) produced significantly higher values than non-grafted plants. Though the non-grafted plant of Iraq 5 (0.050) produced significantly lower values. Under drought stress condition, there were no significant differences observed among grafted and non-grafted plants. The non-grafted plant of Iraq 5 (0.077) produced higher values, on the other hand the graft combination of Goldene Königin F1/Karahıdır produced higher (0.048) ANTH content. Decline in ANTH content was rated among –12.12 and 53.3%. Minimum decline was found at Goldene Königin F1/Karahidir (–12.12%), while maximum decline was observed at non-grafted control plant of Iraq 5 (53.3%) (Fig. 1d). STI values were 79% and 95% at NBI, 84% and 104% at CHL content, 114% and 108% at FLAV content, 90% and 126% at ANT content for grafted and non-grafted plants, respectively (Fig. 1a–d). Oxidative and drought stimulated enhanced construction of these secondary metabolites, containing flavonols and anthocyanins, to mitigate outcomes of stress with their strong radical scavenging activity (Nakabayashi et al. 2014a, b). De Clercq et al. (2023) explained that no increased flavonol or anthocyanin content, determined by the optical sensors, was found under reduced irrigation.

3.4 Shoot and root N concentration and total plant N uptake

Shoot and root N concentration and total plant N uptake of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions are presented in Table 3. The results indicated that shoot and root N concentration and total plant N uptake at the end of the growing cycle differed in the nutrient solution under different water supply levels. The shoot and root N concentration and total plant N uptake were significantly ($p < 0.001$) affected by grafting, drought and also the interaction of grafting × drought stress. Considering grafting, generally at the hydroponic experiment under drought stress condition, the grafted and non-grafted tomato plants produced lower shoot and root N concentration and total plant N uptake as compared to control condition. Increasing drought stress at the hydroponic condition decreased shoot N concentration by almost 9.30%, root N concentration by almost –51.25%, and the total plant N uptake by almost –60.56% than plants grown under control condition. Shoot N concentration values ranged between 22.69 and 44.39 mg g^{-1} under control conditions; however, it was 29.08–47.80 mg g^{-1} under drought stress. Under control conditions root N concentration values ranged among 8.58–87.11 mg g^{-1}, under drought stress it was 16.97–21.80 mg g^{-1}. Regarding total plant N uptake, the values ranged linking 39.44–2769.5 mg N $plant^{-1}$ under control conditions; under drought conditions it was 340.8–2054.2 mg N $plant^{-1}$. Up to now in many studies, it was observed that environmental stresses might substantially influence shoot and root N concentration and total plant N uptake. Similar results also observed by Adam and Ulas (2023) at tomato plants, Ulas et al. (2019a, b) at watermelon plants, Ulas et al. (2021a) at potato plants, and Ulas et al. (2021b) at pepper plants.

Vegetable grafting has been chosed in many crop species to prevent drought (Ulas et al. 2019a, b; Rekaby et al. 2021; El-Mogy et al. 2022). In some research papers, it has been stated that drought tolerance can enhance with grafting (Liu et al. 2014b; Koevoets et al. 2016; Shehata et al. 2022). Grafting enhanced shoot N concentration by –24.65% and

Table 3 Shoot and root N concentration and total plant N uptake of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions

Grafted combinations	Shoot N Conc [mg g^{-1}]			Root N Conc [mg g^{-1}]			Total N Uptake [mg N $plant^{-1}$]		
(S/ R)	Control	Drought	R%	Control	Drought	R%	Control	Drought	R%
Iraq 5	44.39 a	45.88 B	3.4	40.22 b	21.80 A	−45.8	2770 a	688 B	−75.2
Iraq 5/ALT	22.69 c	41.83 C	84.4	87.11 a	17.77 C	−79.6	2144 c	2054 A	−4.2
GK	41.49 b	47.80 A	15.2	21.38 c	16.97 D	−20.6	2396 b	698 B	−85.8
GK/Karahidir	42.02 b	29.08 D	−30.8	8.58 d	20.15 B	134.8	2275 b	341 C	−69.3
STI (%)	109.3			48.75			39.44		
F-Test									
Graft.Comb	***			***			***		
Drought	***			***			***		
Graft. Comb. × Drought	***			***			***		

Values denoted by different letters (lower and upper case letters for control and drought stress, respectively) are significantly different between genotypes within columns at $p < 0.05$. *ns* non-significant. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. GK: Goldene Königin F1, *R* reduction, *S* scion, *R* Rootstock

–24.30%, root N concentration by 55.34% and –2.17% and total plant N uptake by –14.46% and 167.61% at control and drought conditions, respectively than control plants. Drought stress causes a decline in shoot N concentration between –30.80% and 84.38%, root N concentration between –20.63% and 134.76%, and total plant N uptake between –85.77% and –4.17% depending on the rootstocks. Minimum decline was found at Goldene Königin F1/Karahidir (–30.80%), whilst maximum decline was found in Iraq 5/ALT (84.38%) at shoot N concentration. Under control conditions, non-grafted Iraq 5 (44.39 mg g^{-1}) produced significantly higher shoot N concentration, though non-grafted Goldene Königin F1 (47.80 mg g^{-1}) produced significantly higher shoot N concentration under drought stress. Regarding root N concentration, the significantly higher values were produced by Iraq 5/ALT (87.11 mg g^{-1}) under control conditions. On the other hand, non-grafted Iraq 5 (21.80 mg g^{-1}) produced significantly higher root N concentration under drought stress. The minimum reduction was observed in the graft combination of Iraq 5/ALT (–79.60%), while the maximum reduction was observed in Goldene Königin F1/Karahidir (134.76%) at root N concentration. Under control conditions, non-grafted Iraq 5 (2769.5 mg N $plant^{-1}$) produced significantly higher total plant N uptake, though the graft combination of Iraq 5/ALT (2054.2 mg N $plant^{-1}$) produced significantly higher total plant N uptake under drought stress. The minimum reduction was observed in the non-grafted Goldene Königin F1 (–85.77%), while the maximum reduction was observed in Iraq 5/ALT (4.17%) at total plant N uptake. STI values were 109.3% at shoot N concentration, 48.75% at root N concentration and 39.44% at total plant N uptake (Table 3). The above stated results confirm with the findings of Adam and Ulas (2023) which indicates that increase in nitrogen supply had significant impacts on NRA activity and shoot N uptake and shoot N concentration at tomato plants. Ruiz and Romero (1999), examining N metabolism in grafted melon plants, found that practice of confident rootstocks in squash increased NO_3^- reduction and N use, defined as a rise in leaf organic N content, which ultimately boosted production. Conversely, reply of grafted melon to N was examined at one level of nitrate in nutrient solution (6 mM of NO_3), however report was missing regarding influence of different supplies of nitrate on grafted melon crop performance. The outcomes of Colla et al. (2010) stated that grafted melon plants have greater N uptake and N utilization than non-grafted plants. Grafted plants which have a compatible rootstock demonstrate well absorption of nutrient and water and utilize nutrients very efficiently owing to rootstock vigorous root system that transfers water and nutrients better to scion (Gregory et al. 2013; Albacete et al. 2015).

3.5 Nitrogen uptake effectiveness (NUP), nitrogen use efficiency (NUE) and biological production efficiency (BPE) in shoot

In general Nitrogen Uptake Effectiveness (NUP), Nitrogen Use Efficiency (NUE) and Biological Production Efficiency (BPE) in shoot was negatively affected by increasing the drought stress of the nutrient solutions. NUP, NUE and BPE were significantly ($p < 0.001$) impacted by grafting, drought stress and also grafting × drought stress interaction (Table 4). Regardless of grafting, under drought stress condition, NUP, NUE and BPE of the grafted and non-grafted tomato plants were lower than plants grown under control condition. Increasing drought stress at the hydroponic condition decreased NUP by almost –59.88%, NUE by almost –61.58%, and BPE by almost –11.77% than plants grown under control condition. These findings coincide with those of Hoang et al. (2019) and Consentino et al. (2023) who found that water constraints decrease NUE. Martínez et al.

Table 4 Shoot Nitrogen Uptake Effectiveness (NUP), Shoot Nitrogen Use Efficiency (NUE) and Shoot Biological Production Efficiency (BPE) of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions

Grafted combinations	Shoot N Uptake Efficiency			Shoot N Use Efficiency			Shoot Biological Production Efficiency		
(S/ R)	Control	Drought	R%	Control	Drought	R%	Control	Drought	R%
Iraq 5	73.58 a	18.37 B	−75.0	1657 a	400 C	−75.9	22.53 c	21.80 C	−3.3
Iraq 5/ALT	34.05 c	50.35 A	47.9	1499 a	1202 A	−19.8	44.08 a	23.90 B	−45.8
GK	68.00 b	9.58 C	−85.9	1638 a	200 D	−87.8	24.10 b	20.92 C	−13.2
GK/Karahidir	66.39 b	18.79 B	−71.7	1580 a	646 B	−59.1	23.80 b	34.41 A	44.6
STI (%)	40.12			38.42			88.23		
F-Test									
Graft. Comb	***			***			***		
Drought	***			***			***		
Graft. Comb. × Drought	***			***			***		

Values denoted by different letters (lower and upper case letters for control and drought stress, respectively) are significantly different between genotypes within columns at $p < 0.05$. *ns* non-significant. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. *GK* Goldene Königin F1, *R* reduction, *S* scion, *R* Rootstock

(2017) stated maximum NUP at 0 kg N ha^{-1} N level, however minimum NUP was stated at 300 kg N ha^{-1} N level, and their average NUP fluctuated from 0.59 to 0.83 kg kg^{-1} For maize with seven N application amounts under sprinkler irrigation in a petrocalcic calcixerept soil in northeastern Spain. Arregui and Quemada (2008) found in wheat and barley much lower NUP data, vary between 0.34 and 0.43 kg kg^{-1} with negligible fertilizer splitting and nitrification inhibitor effects. They assigned their low NUP data to N losses from fertilizer and nitrate–N leaching among planting and first N application date. Quemada and Gabriel (2016) stated NUP vary between 0.55 and 0.8 kg kg^{-1} for sprinkler-irrigated maize. Paponov et al. (1996) and Masclaux-Daubresse et al. (2010) found that NUE was definitely linked with enhance in biomass or grain yield per unit of N uptake. In a similarity among conservation and not ill practices for maize, Habbib et al. (2016) found that NUE data were considerably ($p < 0.05$) greater in not ill than conservation tillage, and their values ranged from 21 to 52 kg kg^{-1}. They stated that NUE was positively linked with enhance in biomass or grain yield per unit of N uptake. Liu et al. (2019) stated maize NUE data fluctuating from 40 to 60 kg kg^{-1}, and they observed that late harvest (6–10 days later than a conventional/ long-term average day) encouraged NUE considerably ($p < 0.05$) by an average of 4.5% for three different late harvest dates and two growing seasons.

Grafting had substantial positive influences and consequently, grafted plants demonstrated considerably greater NUP, NUE and BPE than non-grafted plants grown at both control and drought stress conditions. Grafting increased NUP by –29.06% and 147.45%, NUE by –6.60% and 207.86%, BPE by –2.03% and –37.93% at control and drought conditions, respectively than control (non-grafted) plants. Regarding NUP, under control conditions, considerably greater data were observed by non-grafted tomato plants of Iraq 5; though under drought stress condition, considerably greater NUP was produced when the tomato scion genotype of Iraq 5 was grafted on to ALT. The reduction in the shoot NUP was ranked between –85.92 and 47.90%. Minimum decline was found in Goldene Königin F1 (–85.92%), while the maximum reduction was observed in graft combination of Iraq 5/ ALT (47.90%) (Table 4). The uptake efficiencies of N were significantly increased in apple scions grafted on M9 rootstock (Amiri et al. 2014). Grafted on to the 'Gisela 6' rootstock of sweet cherry results in high uptake and translocation efficiencies for N (Nawaz et al. 2016). Concerning NUE, under control conditions, there were no significant differences observed among the non-grafted and grafted tomato plants. Though under drought stress condition, the significantly higher NUE was produced when the tomato scion genotype of Iraq 5 was grafted on to ALT. The reduction in the shoot NUE was ranked between –87.78 and –19.76%. The minimum reduction was observed in Iraq 5 (–87.78%), while the maximum reduction was observed when the tomato scion genotype of Iraq 5 was grafted on to ALT (–19.76%) (Table 4). Wang et al. (2022) stated that sweet pepper yields tend to rise quadratically with increasing irrigation, and either too high or too low irrigation inhibits yield formation and reduces water and nitrogen use efficiency. In terms of BPE, the significantly higher values were produced at the graft combination of Iraq 5/ ALT under control condition; on the other hand, the significantly lower values were observed at the non-grafted plant of Iraq 5 under control condition. Under drought stress condition, the graft combination of Goldene Königin F1/Karahidir (34.41) produced significantly higher values. It was followed by the graft combination of Iraq 5/ ALT (23.90). However, the non-grafted plants of Iraq 5 (21.80) and Goldene Königin F1 (20.92) produced significantly lower values. The reduction in the shoot BPE was ranked between –45.77 and 44.59%. The minimum reduction was observed when the tomato scion genotype of Iraq 5 was grafted on to ALT (–45.77%), while the maximum reduction was observed when the tomato scion genotype of Goldene Königin F1 was grafted on to Karahidir (44.59%). STI values were 40.12% at NUP, 38.42% at NUE, 88.23% at BPE for grafted and non-grafted plants (Table 4). Previous studies have shown that in grassland plants, drought will reduce NUE (i.e., C/N) (Sun et al. 2020; Luong et al. 2021; Guo et al. 2022), like *Leymus chinensis* (Yue et al. 2019) and six common grassland plant species (*Leymus chinensis, Stipa baicalensis, Thermopsis lanceolata, Potentilla bifurca, Thalictrum squarrosum, Klasea centauroide*) (Hu et al. 2023).

3.6 Shoot macro and micro element analysis

The shoot macro and micro element concentrations and distributions in grafting combinations and tomato plants as a function of drought stress were shown in Table 5. The results indicated that shoot P (%), K (%), Ca (%), Mg (%), S (%) and Fe (ppm) concentrations significantly ($p < 0.001$) affected by grafting, drought and the interaction of grafting × drought stress. Increasing drought stress at the hydroponic condition decreased significantly the P, K, Ca, Mg, S and Fe concentrations in shoot. The significantly highest values for the concentrations of P (0.74–0.68%), Ca (3.12–3.19%), Mg (0.93–0.76%), S (1.92–1.78%) and Fe (287.2–116.25 ppm) in shoot recorded by the grafting combination of Iraq 5/ALT under both control and drought stress conditions, respectively. The significantly higher shoot K concentration was observed by the non-grafted Goldene Königin F1 under both control and drought stress conditions. Under control conditions shoot P concentration values ranged between 0.59 and 0.74%; however, it was 0.46–0.68% under drought stress. Moreover, under drought stress, the shoot P concentration ranged from 0.68 to 0.46% in the non-grafted and grafted

Table 5 Shoot macro and micro element analysis of non-grafted and grafted tomato cultivars grown at high N supply (3.0 mM) under drought stress conditions

Grafted combinations		Leaf macro and micro element contents					
		P	K	Ca	Mg	S	Fe
(S/R)	Treatment	%	%	%	%	%	ppm
Iraq 5	Control	0.72 b	3.63 d	2.83 b	1.55 b	141.5 b	141.6 b
	Drought	0.49 C	3.67 D	2.13 B	0.62 C	0.74 B	45.86 B
	Mean	0.61	3.65	2.13	0.73	1.15	93.75
	R%	−31.88	1.24	−24.88	−25.04	−52.21	−67.62
	STI (%)	68.12	101.24	75.12	74.96	47.79	32.38
Iraq 5/ALT	Control	0.74 a	4.16 b	3.12 a	0.93 a	1.92 a	287.2 a
	Drought	0.68 A	3.75 C	3.19 A	0.76 A	1.78 A	116.25 A
	Mean	0.71	3.95	3.15	0.84	1.85	201.7
	R%	−7.03	−9.92	2.09	18.04	−6.99	−59.52
	STI (%)	92.97	90.08	102.09	81.96	93.01	40.48
GK	Control	0.67 c	4.40 a	2.34 c	0.75 c	1.28 c	93.74 c
	Drought	0.58 B	3.93 A	1.93 C	0.65 B	0.68 D	47.26 B
	Mean	0.62	4.16	2.14	0.70	0.98	70.5
	R%	−13.64	−10.54	−17.40	−13.73	−46.38	−49.58
	STI (%)	86.36	89.46	82.6	86.27	53.62	50.42
GK/Karahidir	Control	0.59 d	4.14 c	2.23 d	0.65 d	1.14 d	102.3 c
	Drought	0.46 D	3.80 B	1.50 D	0.46 D	0.70 C	45.09 B
	Mean	0.53	3.97	1.87	0.56	0.92	73.69
	R%	−21.41	−8.27	−32.46	−28.68	−38.55	−55.92
	STI (%)	78.59	91.73	67.54	71.32	61.45	44.08
F-Test	Graft. comb	***	***	***	***	***	***
	Drought	***	***	***	***	***	***
	Graft. comb. × Drought	***	***	***	***	***	***

Values denoted by different letters (lower and upper case letters for control and drought stress, respectively) are significantly different between genotypes within columns at $p < 0.05$. *ns* non-significant. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. *GK* Goldene Königin F1, *R* reduction, *S* scion, *R* Rootstock

plants, respectively. STI values of shoot P concentration was 92.97% at Iraq 5/ALT graft combination, 86.36% at Goldene Königin F1, 78.59% at Goldene Königin F1/ Karahidir graft combination, and 68.12% at Iraq 5, respectively. Compared to the non-stressed plants, drought stress increased the shoot P concentration of the Iraq 5 and Iraq 5/ALT plants by –31.88 and –7.03%, respectively. Parallel outcomes were observed for leaf P concentration at watermelon (Colla et al. 2010; Uygur and Yetisir 2009) and tomato (Fernandez-Garcıa et al. 2004) plants via grafting, determining that graft combination can affect P absorption positively. Though, Kawaguchi et al. (2008) stated that rootstock species was major influence altering absorption and translocation of this element in graft combinations of Solanaceous crops. Under control conditions shoot K concentration values ranged between 4.40 and 3.63%; however, it was 3.93–3.67% under drought stress. Averaged shoot K concentration showed variation across tomato plants from about 3.93 to 3.67% in grafted and non-grafted plants under drought stress, respectively. STI values of shoot K concentration was 101.24% at Iraq 5, 91.73% at Goldene Königin F1/ Karahidir graft combination, 90.08% at Iraq 5/ALT graft combination, and 89.46% at Goldene Königin F1, respectively. The reduction in the shoot K concentration was ranked between –10.54 and 1.24%. The minimum reduction was observed in Goldene Königin F1 (–10.54%), while the maximum reduction was observed in Iraq 5 (1.24%). Related outcomes have been stated in tomato (Fernandez-Garcıa et al. 2004; Sadeghi et al. 2023) and watermelon (Goreta et al. 2008), confirming effectiveness of grafting on K absorption by rootstock and translocation to scion. Under control conditions shoot Ca concentration values ranged between 2.23 and 3.12%; however, it was 1.50–3.19% under drought stress. STI values of shoot Ca concentration was 102.09% at Iraq 5/ALT graft combination, 82.6% at Goldene Königin F1, 75.12% at Iraq 5, and 67.54% at Goldene Königin F1/ Karahidir graft combination, respectively. Under drought stress, the shoot Ca concentration ranged from 3.19 to 1.50% in the non-grafted and grafted plants, respectively. Decline in shoot Ca concentration was ranked between –32.46 and 2.09%. Minimum decline was found in Goldene Königin F1/ Karahidir (–32.46%), though maximum decline was found in Iraq 5/

ALT (2.09%). It has been found that Ca accumulation is restricted by xylem transport (Ho et al. 1987) and any interruption in xylem pathway may affect Ca movement from root to shoot. It can be recommended that a lower Ca concentration in grafted crops in comparison with control may be linked negative role of graft junction on water flow through xylem vessels. Under control conditions shoot Mg concentration values ranged between 0.65 and 0.93%; however, it was 0.46–0.76% under drought stress. Averaged shoot Mg concentration showed variation across tomato plants from about 0.76 to 0.46% in grafted and non-grafted plants under drought stress, respectively. STI values of shoot Mg concentration was 86.27% at Goldene Königin F1, 81.96% at Iraq 5/ALT graft combination, 74.96% at Iraq 5, and 71.32% at Goldene Königin F1/ Karahidir graft combination respectively. The reduction in the shoot Mg concentration was ranked between –28.68 and 18.04%. The minimum reduction was observed in Goldene Königin F1/ Karahidir graft combination (–28.68%), while the maximum reduction was observed in Iraq 5/ALT graft combination (18.04%). Under drought stress, the shoot S concentration ranged from 1.78 to 0.68% in the non-grafted and grafted plants, respectively. STI values of shoot S concentration was 93.01% at Iraq 5/ALT graft combination, 53.62% at Goldene Königin F1, 61.45% at Goldene Königin F1/ Karahidir graft combination, and 47.79% at Iraq 5, respectively. Under control conditions shoot S concentration values ranged between 1.14 and 1.92%; however, it was 0.68–1.78% under drought stress. Compared to the non-stressed plants, drought stress increased the shoot S concentration of Iraq 5 and Iraq 5/ALT plants by –52.21 and –6.99%, respectively. Under control conditions shoot Fe concentration values ranged between 93.74 and 287.2 ppm; however, it was 45.09–116.25 ppm under drought stress. Averaged shoot Fe concentration showed variation across tomato plants from about 116.25 to 45.09 ppm in grafted and non-grafted plants under drought stress, respectively. STI values of shoot Fe concentration was 50.42% at Goldene Königin F1, 44.08% at Goldene Königin F1/ Karahidir graft combination, 40.48% at Iraq 5/ALT graft combination, and 32.38% at Iraq 5, respectively. The reduction in the shoot Fe concentration was ranked between –67.62 and –49.58%. The minimum reduction was observed in Iraq 5 (–67.62%), while the maximum reduction was observed in Goldene Königin F1 (–49.58%) (Table 5). Due to the robust root system utilised as the rootstock, grafted plants typically exhibit enhanced uptake of water and nutrients compared to non-grafted plants (Sánchez-Rodríguez et al. 2012). Sadeghi et al. (2023) examined seven Solanaceous species (*Solanum pimpinellifolium, Solanum melongene* cv. Thai yellow, *Solanum lycopersicum* cv. Emperador, *Solanum melongene* cv. Mohalli Yazd, *Solanum cheesmaniae*, *Solanum nigrum*, *Solanum lycopersicum* var. *cerasiforme*) as rootstock to evaluate the performance of Daphnis cultivar to mild, moderate and severe drought stress. The outcomes displayed that N, Ca and Mg concentration in leaf and root were not affected by irrigation intervals, whereas P was enhanced in leaf and root with improving irrigation intervals and K was enhanced in leaf and reduced in root. The concerned rootstocks had an obvious influence on growth, yield, P and K concentration in leaf and root. Cherry tomato (*Solanum lycopersicum* var. cerasiforme) was superior than other rootstocks in terms of examined parameters, followed by Galapagus and Emperador. The convinced function of these rootstocks in mitigation of harmful impacts of drought may be linked to their vigorous root system. It is established that grafting is a healthy practice, because no chemical management is needed, may enhance resistance of tomato plants against drought depending on rootstock genotype. Cabrera De la Fuente et al. (2023) studied influence of anchor point (soil and substrate), graft (with and without graft), and irrigation volume (100 and 75%) on fruit quality, macro and microelement content, and enzymatic antioxidant activity. Outcomes stated that while there was a higher accumulation of potassium due to graft, there was no difference for calcium. Graft correspond to a possible option for effective use of water, in soil and substrate, being substrate with best environment for development, mitigating stress by reducing enzymatic activity.

4 Conclusion

Due to insufficient nitrogen (N) fertilizer application and water supply by small-scale farmers, soil fertility is declining in low-input agriculture. On the other hand, the concerns are rising on regarding the environmental pollution of both air and water in high-input agriculture due to excessive use of N fertilizer. To improve nitrogen use efficiency (NUE) and drought tolerance of tomato crops for a sustainable agriculture, combined N and water management strategies that take into consideration improved fertilizers, soil and crop water management practices are necessary. Therefore, the aim of this study was to determine genotypic differences in NUE of some local and hybrid tomato genotypes and to assess whether grafting could improve the NUE and drought tolerance of susceptible tomato scions through examining the changes induced by the rootstocks in root morphological, shoot agronomical, leaf physio-biochemical responses at high N level (3.0 mM) under drought stress (10% (w/v) PEG 6000) treated hydroponic condition. Two tomato genotypes [Goldene Königin F1 (German hybrid variety) and Iraq 5 (obtained from Iraq Crop Research Institute)] were used as scion and two pre-selected tomato genotypes [Karahidir (N-inefficient Turkish local variety) and ALT (N-efficient Ghanaian local variety obtained from Ghana Research Institute] were used as rootstock materials in this study.

Although growing under high N supply, shoot growth, as well as root morphological, leaf physiological and biochemical responses and NUE of both grafted and non-grafted (scions) tomato plants were significantly adverse affected by drought stress. On the other hand, the grafted plants usually exhibited highest growth performance than the non-grafted control plants under drought stress indicating that N-efficient and drought tolerant rootstocks significantly contributed to growth and biomass production and thus NUE of susceptible tomato scions. Among the two graft combinations significant genotypic variation existed in drought stress under high N supply. Previously as 'N-efficient' characterized tomato root stock genotype ALT showed higher drought tolerance in the graft combination of Iraq 5/ALT than the 'N-inefficient' characterized genotype KH in the graft combination of GK/KH. Two non-grafted susceptible scion genotypes varied significantly under drought stress. The scion genotype Iraq 5 usually showed a higher drought tolerance than the scion genotype GK under high N supply. Both rootstocks (ALT and KH) have high rootstock potential for tomato, and their significant contributions to salt tolerance and to NUE were closely associated with inducing physiological and biochemical responses of scions under high N supply. These traits could be useful for the selection and breeding of drougth-tolerant rootstocks for sustainable high-input agriculture in the future. However, to recommend these tomato rootstocks for using in a low-input agriculture, the experiment should be conducted also under a low N condition. Therefore, a next hydroponic study will be conducted by grafting same tomato scions (Iraq 5 and GK) onto the same rootstocks (ALT and KH) under low N (0.3 mM) supply which will be treated with drought stress in the near future.

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Data availability All of the data supporting the findings of this study are included in this article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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Consent to publication All authors agreed to publish the article.

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