

ACCEPTED ARTICLE

Drought stress effects on the aerial part physiology and macronutrient uptake in pear (*Pyrus communis* L.) scion-rootstock combinations

Zeinab Maleki Asayesh¹, Kazem Arzani^{2*}, Ali Mokhtassi-Bidgoli³, Hamid Abdollahi⁴

¹Graduated Ph.D. student, Department of Horticultural Science, Tarbiat Modares University, Tehran, Iran

²Professor, Department of Horticultural Science, Tarbiat Modares University, Tehran, Iran

³Assistant Professor, Department of Agronomy, Tarbiat Modares University, Tehran, Iran

⁴Associate Professor, Temperate Fruits Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

*Corresponding Author: arzani_k@modares.ac.ir

Abstract

Drought is a major abiotic stress, especially in arid and semi-arid regions. To determine the optimized scion-rootstock combination, research can focus on the physiological characteristics of their aerial part as well as its capacity to uptake macronutrients in response to drought stress. The effect of drought stress and the rootstock with different root systems, on gas exchange, uptake, and use of macronutrients, and osmolytes were assessed on two-years-old seedlings of two pears (*Pyrus communis* L.) cultivars, 'Dargazi' and 'Louise Bonne' grafted onto 'Dargazi' seedlings and 'Pyrodwarf' clone rootstocks in the present study. Treatments were well-watered as control (100% Field capacity, FC), moderate (60% FC), and severe drought (30% FC). Results showed that root-to-shoot ratio (*R/SH ratio*), leaf relative water content (*LRWC*), soluble protein (*SP*), photosynthetic nitrogen use efficiency (*PNUE*), leaf osmotic potential (Ψ_s), leaf potassium (LK^+), root calcium (RCa^{2+}), root nitrogen (*RN*), stomatal aperture (*SA*), and gas exchange traits such as net photosynthetic rate (*An*), transpiration rate (*E*), stomatal conductance (g_s), instantaneous carboxylation efficiency of Rubisco (*EiC*), of all scion-rootstock combinations decreased by

drought stress. In addition, proline (*Prol*), glycine betaine (*GB*), total soluble carbohydrate (*TSC*), root potassium (RK^+), leaf calcium (LCa^{2+}), leaf nitrogen (*LN*), leaf phosphorus (*LP*), and root phosphorus (*RP*) were increased under drought stress conditions. Under severe levels of stress, the 'Dargazi' as the cultivar or rootstock exhibited better instantaneous water use efficiency (*WUEi*), nitrogen utilization efficiency (*NUtE*), and nitrogen uptake efficiency (*NUpE*), as well as higher contents of *LN*, LNO_3^- , LNH_4^+ , *Prol*, and Ψ_s . On the other hand, 'Louise Bonne' showed higher amounts of *R/S* ratio, total nitrogen accumulation (*TNA*), leaf and root *NRA*, *An*, intracellular CO_2 concentration (*Ci*), *E*, and root nitrate (RNO_3^-) under moderate stress levels. In addition, the 'Pyrodwarf' rootstock showed the highest *TNA* and RCa^{2+} under moderate stress level conditions. The present obtained results suggested that under severe stress levels, 'Dargazi' showed better tolerance as the seedling rootstock or cultivar, and 'Pyrodwarf' clonal rootstock and 'Louise Bonne' cultivar had better response under moderate stress conditions.

Keywords: Abiotic stresses, Macronutrients, Osmolytes, Pear, Photosynthesis, Scion-rootstock.

1. Introduction

Fruit trees, unlike other plants, are generally composed of two different parts, the rootstock and the scion growing together as one. In general, rootstocks as the tree's root system play an important role, in influencing the whole plant's growth and development. Besides, nutrient uptake is adjusted by its availability on the plant's root surface, which depends on the amount and movement of water into the soil (Li *et al.*, 2013; Martinez *et al.*, 2020). The stomata affect the plant–water relations and gas exchange, and drought stress can reduce nutrient uptake by the root, nutrient transport from the root to the shoot, and partitioning in plants due to a restricted transpiration rate, limited active transport, and reduced membrane permeability (Asayesh *et al.*, 2023b; Clavijo-Herrera *et al.*, 2018; Sanchez-Rodriguez *et al.*, 2014). In addition, Drought stress influences the net carbon assimilation rate, chlorophyll pigments, and photosynthesis, either by a direct effect of dehydration on the photosynthetic apparatus or by an indirect effect by way of stomatal closure (Xia *et al.*, 2020). In plants, the transpiration rate determines the amount of calcium (Ca^{2+}) and potassium (K^+) absorbed by the leaf (de Freitas and Mitcham, 2012). The osmotic adjustment (OA), as a tool that lowers osmotic potential due to net solute accumulation in response to drought stress, by the conservation of turgor during plant's water status changes may help to preserve the metabolic

processes and contribute to growth in plants (Martinez *et al.*, 2004). Drought can hamper many plant physiological processes under suboptimal nitrogen concentration. It often results in a decrease in the uptake of NH_4^+ and NO_3^- because of the plant's less available energy for their assimilation (Huang *et al.*, 2018a). Therefore, improving the plant's *WUE* and *NUE* under drought stress conditions led to improved physiological acclimation to drought conditions (Wei-Feng and Yu-Zheng, 2014).

European pear (*Pyrus communis* L.) is one of the most important commercial fruit tree species that grow well in the temperate zones of the world and also in Iran (Arzani, 2021). The careful selection and use of the seedling or clonal rootstock and the scion to rootstocks combinations are critical for a better or proper orchard management system to lead to optimum crop yield, especially under unfavorable environmental conditions (Arzani, 2017; Karbasi and Arzani, 2021). Therefore, given the interactions that exist between the rootstock, the cultivar, and the environment, the selection of scion-rootstock combinations that are affected by the rootstock root system is an important task for a better orchard management system (Arzani, 1994; Arzani, 2017). Several previous reports demonstrated the relationships between various important physiological parameters of pear scion-rootstock combinations which can influence water relations, leaf gas exchange, blooming, fruit set and quality, plant size, and yield efficiency (Asayesh *et al.*, 2023b; Babaei *et al.*, 2021; Bosa *et al.*, 2014; Ikinci *et al.*, 2014). Trees enhancement vigor by the root system of rootstock and its effect on plant yield could be increased water and nutrient uptake in grafted plants. The mineral content value in aerial parts of the plant was attributed to the physical characteristics of the root system such as lateral and vertical development in the rootstock, which is one of the main reasons for the widespread use of grafted rootstocks (Sanchez-Rodriguez *et al.*, 2014; Seki *et al.*, 2007). Although cultivation of fruit crops is being shifted to the clonal rootstocks in most fruit-growing countries, seedling rootstocks because of their deep root might be more suitable rootstocks to be used under unfavorable growing conditions such as drought situations (Arzani, 2017; Karbasi and Arzani, 2021). Besides, every rootstock responds differently to various agro-climatic conditions, therefore, further study of clonal compared to seedling rootstocks is necessary (Asayesh *et al.*, 2023a).

In addition, some aspects of 'Pyrodwarf' as a semi-dwarfing clonal rootstock, and 'Dargazi' as a popular and more commonly available seedlings rootstock for nutrient uptake efficiency were reported by Asayesh *et al.*, 2022. Nevertheless, as far as we know, knowledge about the effects on

nutrient uptake and the consequences for physiological processes is scarce, especially on the scion to rootstock combinations under drought-stress conditions. Therefore, this research was investigated to get a better understanding of the relationship between stomatal movement and gas exchange in the aerial part of pear plants with macronutrient uptake and use under drought stress in four different pear scion-rootstock combinations that were grafted on two rootstocks by the different root system. The aim which can help to specify the efficacy of rootstocks on plant nutrient status to optimize fertilization programs and better management of pear orchards under water stress conditions.

2. Material and methods

2.1. Plant material

We obtained two-year-old pear trees (*Pyrus communis* L.) from a commercial nursery in Karaj, Iran. The trees were developed from 'Dargazi' (D) seedlings and in vitro propagated semi-dwarf clonal 'Pyrodwarf' (P) rootstocks. The mature buds of commercially important scion cultivars, 'Dargazi' and 'Louise Bonne' (L), were grafted earlier onto the rootstocks, resulting in four different rootstock-scion cultivar combinations (L/D, D/D, L/P, and D/P). All of the cultivars and rootstocks are economically important locally. The developed trees were transferred into 12 L pots (one plant per pot) during the dormant season, filled with loamy soil with the following characteristics: pH: 7.59, EC: 2.3 ds.m^{-1} , available nitrogen: 0.24%, available phosphorus: 110 mg kg^{-1} , and available potassium: 717.6 mg kg^{-1} . The experiment was carried out under the research greenhouse conditions, Department of Horticultural Science, Tarbiat Modares University, Tehran, Iran. The research greenhouse received natural sunlight with a 10 to 14-hour photoperiod, 34/24 °C, and 45/60% average day/night temperature and relative humidity (%RH), respectively.

2.2. Drought treatment application

The experimental trees were allowed to grow for four months in the greenhouse and well-watered conditions. Then, trees were subjected to the applied stress treatments for 60 days (August to September 2020). The field capacity (FC) of the soil was determined by using the laboratory pressure plate method (Ferrer *et al.*, 2008). The irrigation treatments were well-watered as control (100% FC), moderate drought stress (60% FC), and severe drought stress (30% FC) with the soil moisture content of 34.49%, 20.69%, and 10.34% after irrigation, respectively. To minimize the surface evaporation effect, five pots without plants were used to determine evaporative water loss

from the soil surface throughout the 60-day experimental period. Water loss was evaluated gravimetrically by weighing all pots and differences in weight that occurred between the weight of re-watered pots and their weight after 48 h, were calculated. Then the amount of water lost via evaporation was added to each pot during irrigation.

All measurements and sampling were performed at the end of the experiment (60 days after the start of the drought stress treatment).

2.3. *Morpho-physiologic characteristics*

At the end of the experiment (60 days after the start of the drought stress treatment), trees were destructively harvested and the shoot and root samples were dried in an electric oven at 70 °C, total dry weight (*TDW* (shoot + root) and root-to-shoot ratio (*R/SH*) was recorded.

The chlorophyll index was recorded using a SPAD chlorophyll meter (CCM-200, Opti-Sciences, Inc., Hudson, NH, USA). Leaf relative water content (*LRWC*) was determined by the method described by (Arzani, 1994; Wu *et al.*, 2008) as the following formula: $LRWC (\%) = (FW - DW) / (TW - DW) \times 100$. Where FW is the fresh weight, TW is the turgid weight after rehydrating samples for 24 h, and DW is the weight recorded after oven-drying samples at 85°C for 24 h.

2.4. *Ψ_s and compatible solutes*

Leaf osmotic potential (Ψ_s) was determined according to (Martinez *et al.*, 2004), by using collected leaf sap. Osmolarity was assessed with a vapor pressure osmometer (Osmomat 030 Gonotec), and converted from mOsmole kg⁻¹ to MPa according to the Van't Hoff equation.

The proline concentration of leaves was spectrophotometrically measured as described by (Bates *et al.*, 1973). The absorbance of free proline content (μg g⁻¹ FW) was read at 520 nm, and calculated based on a standard curve prepared with five standard concentrations (0–200 μg mL⁻¹) of L-proline.

Glycine betaine (GB) content (μg g⁻¹DW) was estimated according to the method of (Grieve and Grattan, 1983). The absorbance was determined at 365 nm with a spectrophotometer (SmartSpec Plus Spectrophotometer, BIO-RAD), and glycine betaine content was measured according to a standard curve drawn from 0–200 μg mL⁻¹ glycine betaine as prepared in 2 M sulfuric.

2.5. *TSC and SP*

Total soluble carbohydrates (TSC) were determined by the anthrone and reading absorbance at 625 nm based on the method described by (Vandoorne *et al.*, 2012). To quantify the total soluble carbohydrates (mg g^{-1} FW) a calibration curve was established using glucose as the standard.

The soluble protein (SP) content was determined by the G-250 reagent (Bradford, 1976). Then the protein content (mg g^{-1} FW) was calculated according to reference standards of bovine serum albumin (BSA).

2.6. Stomatal morphology

To investigate the differences in stomatal morphology between pear scion-rootstock combinations, the stomata of the lower epidermis on the new fully expanded leaf (abaxial surface) from 4 randomly selected leaves and 80 stomata taken from each leaf were investigated by using a light microscope (model Olympus BX51), according to the method described by (Asayesh *et al.*, 2021). Finally, ImageJ (U.S. National Institutes of Health, Bethesda, MD; <http://imagej.nih.gov/ij/>) was used to measure the stomatal length/stomatal width ratio, stomatal aperture, and stomatal density.

2.7. Gas exchange measurements

The photosynthesis and gas exchange include, net photosynthetic rate (A_n), transpiration rate (E), stomatal conductance (g_s), and intracellular CO_2 concentration (C_i) were measured on the fully expanded young leaves. The gas exchange was recorded between 10:00 am to 2:00 pm on sunny days using a portable photosynthesis system (LI-6400, LI-COR, USA) that setup at 410 ppm CO_2 concentration, 30°C chamber temperature, and the airflow rate of $500 \mu\text{mol s}^{-1}$. The instantaneous water use efficiency (WUE_i) and instantaneous carboxylation efficiency of Rubisco (EiC), were calculated as the ratio between net photosynthesis and transpiration ($WUE_i = A_n / E$) and the ratio between net photosynthesis and intracellular CO_2 concentration ($EiC = A_n / C_i$).

2.8. K^+ , P and Ca^{2+} concentration

To measure the concentration of calcium (Ca^{2+}) and Potassium (K^+) in leaves and roots, 1 g dried leaf sample (70°C for 48 hours), which was extracted with diluted 2 N HCL to extract was used. This extract is used to measure calcium, potassium, and phosphorus contents. The amount of potassium was determined using a flame photometer (model JANEWAY PFP7) and the concentration of calcium was determined by an atomic absorption spectrometer (model GBC 932

AA). To measure phosphorus (P) content in leaves and roots, the colorimetric method of molybdate-vanadate was used by spectrophotometers (model Optizen 3220UV). For this purpose, 2 ml of the extract prepared with ammonium molybdate-vanadate solution reached a volume of 15 ml and was used for reading at 470 nm to determine the amount of phosphorus.

2.9. NO_3^- and NH_4^+ and NRA measurements

The nitrate (NO_3^-) concentration in the dried leaves and roots was determined colorimetrically by the method described by (Singh, 1988). 1 g of samples was digested with 50 ml of 2% acetic acid for 20 min. Then 0.5 g of the powder mixture (citric acid, manganese sulfate monohydrate, sulfanilamide, N-1 naphthyl ethylenediamine dihydrochloride, and powdered zinc) was added to the extract. The reaction mixture was centrifuged and the pinkish-purple color of the supernatant was read at 540 nm.

The ammonium (NH_4^+) content in the roots and leaves was analyzed based on the Berthelot reaction according to (Brautigam *et al.*, 2007). Briefly, 100 mg of fine powder of samples were homogenized in an extraction solution (1 ml 100 mM HCl and 500 μ l chloroform), the extraction solution was shaken for 15 min and then centrifuged at 10,000 g for 10 min at 4°C. The aqueous phase was transferred to a 2 ml tube with 50 mg of activated charcoal and centrifuged at 12,000 g, for 5 min at 4°C. Afterward, NH_4^+ concentration in the supernatant phase was measured at 620 nm.

NRA in leaves and roots was determined according to (Black *et al.*, 2002). Four subsamples of leaf disks (~0.1 g) and roots (~0.2 g) were combined with 5 ml of assay buffer (200 mol m^{-3} KNO_3 and 5% propanol in 100 mol m^{-3} potassium phosphate buffer, pH 7.5) in 20 ml vials. Then placed in the dark at 25°C on a shaker. Two replicate vials for each sample were removed from the shaker after 10 and 90 minutes and placed in boiling water for 15 minutes. To determine nitrite concentration, 500 μ l of 1% sulphanilamide in 3 M HCl and 500 μ l of 0.02% N-naphthyl-ethylene-diamine hydrochloride in water were added to the samples and kept in the dark at room temperature for 20 min, and the absorbance measured at 540 nm with a spectrophotometer (SmartSpec Plus Spectrophotometer, BIO-RAD). Finally, enzyme activity was calculated by comparing the amount of the mean of two replicates of nitrite produced after 90-min incubation with that detected after 10 min and expressed NRA as nmol nitrite produced g (Fresh Mass) $^{-1}$ (fine roots or leaves) h^{-1} (nmol $g^{-1} h^{-1}$).

2.10. Total nitrogen concentration and NUE measurements

The total nitrogen content of oven-dried leaf and root samples was determined by the Kjeldahl distillation method. Samples were distillate with concentrated sulfuric acid and then the Kjeldahl (model UPK132) steam distillation device was used for distilling. Total nitrogen content was used to calculate the different nitrogen use efficiency (*NUE*) definitions (*TNA*, *NUtE*, *NUpE*) according to (Abenavoli *et al.*, 2016), based on the following equations:

- Total Nitrogen Accumulation (*TNA*) = N concentration \times Total plant dry weight (mg N)
- Nitrogen Utilization Efficiency (*NUtE*) = Total plant dry weight / N concentration ($\text{g}^2 \text{TDW mg}^{-1} \text{N}$)
- Nitrogen Uptake Efficiency (*NUpE*) = *TNA* / Root dry weight ($\text{mg N g}^{-1} \text{RDW}$)

Photosynthetic nitrogen-use efficiency (*PNUE*) was calculated according to (Dinh *et al.*, 2017) by the following formula:

- *PNUE* = Photosynthetic rate/Specific leaf nitrogen content

2.11. Statistical analysis

This experiment was conducted as a factorial experiment based on the randomized complete block design (RCBD) with four scion-rootstock combinations, and three levels of drought stress as two factors with four replications for each treatment. The data were subjected to the two-way analysis of variance (ANOVA) to find the significant differences (*P*-value < 0.05) among treatments, and the main effects and their interactions were determined using the general linear model (GLM) procedure of the Statistical Analysis System (SAS 9.2) software. The Duncan multiple comparisons test was performed to compare the means. Interaction and main effects are discussed in the paper in order of their statistical significance, which ranges from the highest-level interactions to the main effects of treatments. When two-way interactions are present for each trait measured, it means that the interpretation of the main effects was incomplete or avoided. At the end of the analysis, the relationship between gas exchange and nitrogen use efficiency parameters was presented by Pearson correlation analysis.

3. Results

3.1. Morpho-physiologic characteristics

In the current study, drought stress had a significant effect on the total dry weight. The *TDW* markedly was different between the scion-rootstock combinations under drought stress conditions. D/P had the highest amount of *TDW* in control and moderate stress levels. Moreover, it decreased

considerably in L/D in moderate stress and D/D and D/P in severe stress compared to control (Figure 3a). A decrease in the *R/SH* ratio was observed in D/D and D/P by increasing stress levels, while L/D (1.3) and L/P (1.2) showed a higher *R/SH* ratio under moderate levels of drought stress (Figure 3b). This indicates that all four scion-rootstock combinations had different responses to drought stress at different levels. The Chlorophyll Index (*SPAD*), in the leaves of four pear scion-rootstock combinations was significantly different. It was higher in D/D and D/P than in L/D and L/P (Figure 1b). While *LRWC* markedly decreased under drought stress levels when compared to that in the control. Reductions in moderate and severe drought stress conditions were 9.4 and 15.63% higher than in control conditions, respectively (Figure 2c).

3.2. Ψ_s and compatible solutes

The Ψ_s were highly affected by scion-rootstock combinations and the interaction of scion-rootstock combinations and drought (Figure 3c). We observed the lowest amount of Ψ_s in L/D between four combinations. Moreover, it decreased considerably under moderate and severe stress conditions, and in L/P and L/D Ψ_s amount in moderate stress was observed lower than severe stress and control (Figure 3c). There was no difference in *GB* content for all four combinations, but an increase was observed in all of them by increasing stress levels. In addition, L/D showed a higher *GB* content under moderate levels of drought stress than in severe (Figure 6a). The concentration of *Prol* was significantly different for drought stress levels and among the four scion-root combinations, D/D and D/P showed more *Prol* accumulation than L/P and L/D (Table 1). In drought stress conditions, the amount of *Prol* increased, this increase in moderate and severe water stress conditions was approximately 13.45 and 25.53% higher than leaf *Prol* in watered conditions (Table 3).

3.3. TSC and SP

An increase in *TSC* content was observed by increasing stress levels, and *TSC* content under severe stress was 11.31 and 20.95% higher than under moderate and control conditions respectively (Figure 2h). The *SP* was affected by drought stress, and its value decreased by approximately 16 and 36% in moderate and severe stress respectively with increasing drought stress (Figure 2g).

3.4. Stomatal morphology

No significant difference between scion-rootstock combinations under drought stress levels was observed for stomatal density (Table 3). The stomatal aperture (SA) was different between the scion-rootstocks combination and decreased with increasing drought stress levels. This reduction was more visible in D/D and D/P combinations (Table 1). In addition, the SA showed a reduction of 12% and 31.64% under moderate and severe stress compared to the control plants (Table 3). We found significant negative correlations between SA and $Prol$ and LCa^{2+} . Besides, there were significant direct correlations between SA with An , g_s , E , and SP (Table 5). The ratio of stomatal length to stomatal width (SL/SW) was increased by drought stress (Table 3), and it was higher for L/P and D/P in comparison with the D/D and D/P combinations (Table 1).

3.5. Gas exchange parameters

The gas exchange parameters showed a significant difference between the drought treatments and four scion-rootstock combinations. A_n was significantly reduced under drought stress, and it was 40.3 and 52% lower in moderate and severe stress levels than the control level (Figure 2a). A huge reduction in g_s was observed in scion-rootstock combinations after 60 days of drought stress. At moderate and severe stress conditions, g_s were approximately two and three times lower than the g_s of control plants (Table 3). Moreover, we found that D/D had the lowest amount of g_s compared to other scion-rootstock combinations (Table 1). A strong reduction in E was observed in four scion-rootstock combinations under drought stress conditions. This reduction, in severe stress compared to the control, was about two times in L/D and D/P and three times in D/D and L/P (Figure 5a). Our results showed a negative significant correlation between An , E , and g_s with $WUEi$, $Prol$, GB , TSC , and LCa^{2+} and a positive relation with SP , LK^+ , and RN (Table 5).

$WUEi$ and EiC factors were significantly different between drought stress levels. $WUEi$ was increased (Table 4) and EiC was decreased (Figure 2i) by increasing drought stress levels. $WUEi$ was 21.88 and 41.13% higher in moderate and severe stress levels than the control level, and the reduction value of EiC in moderate and severe drought stress conditions was 37.7 and 49.18% higher than in control conditions. We observed a negative correlation between the $PNUE$, EiC , and SP with $WUEi$, in contrast, there were significant direct correlations between $WUEi$ with $Prol$, LNO_3^- , and LN (Table 5).

A substantial change in C_i value was observed for all different scion-rootstock combinations with increasing drought stress (Table 2), and a similar trend was observed for WUE_i , with the highest value in D/D (Table 2). The results of different scion-rootstock combinations showed that L/D and D/D had the highest and lowest values of C_i (Table 2). On the other way, with increasing drought stress levels, the amounts of C_i decreased (Table 4). In contrast, the WUE_i value was highest in D/D and lowest in L/D (Table 2), and it increased by increasing drought stress levels (Table 4).

3.6. K^+ , P , Ca^{2+} concentration

According to our results, the drought stress significantly influenced the macronutrient amount in the leaf and root. The levels of LP , RK^+ (Figures 6b and c), RP , and LCa^{2+} (Figures 2e and f), were increased with increasing stress levels. L/D and D/D had the highest amount of RK^+ and the LP content in L/P under moderate and severe stress levels showed a huge increase when compared to control plants. In contrast, the levels of LK^+ and RCa^{2+} were considerably decreased by increasing drought stress levels (Table 2 and Figure 6d). The RCa^{2+} content of L/P and D/P combinations was increased in moderate stress levels and LK^+ content was higher in L/D and D/D combinations. The LK^+ presented a direct correlation with EiC , $PNUE$, and RN , as well as a high negative correlation with LCa^{2+} , LNO_3^- , TSC , LN , and $Prol$ under drought stress conditions (Table 5).

3.7. NO_3^- and NH_4^+ and NRA measurements

The NO_3^- and NRA in leaf and root, were influenced by the scion-rootstock combinations and drought stress levels interactions. The LNO_3^- was increased with increasing drought stress levels and it was more in D/D and D/P in comparison to L/D and L/P (Figure 4b). In contrast, RNO_3^- under moderate stress for L/D, D/D, and L/P changed with an increasing trend, and then all four scion-rootstock combinations significantly decreased under severe stress conditions (Figure 4a). In the case of NRA in both leaf and root, apart from L/D that showed an increase under moderate stress, all four scion-rootstock combinations considerably decreased by stress level increasing. In addition, NRA was different between the four scion-rootstock combinations, and in the control condition, L/P (138) and D/P (141.3) had the highest NRA in root and leaf respectively (Figures 4c and d). The RNH_4^+ was significantly different between scion-rootstock combinations, and it was higher in L/D and L/P than in D/D and D/P. and it was lower under severe drought levels in

comparison with moderate and control conditions (Tables 3 and 5). The LNH_4^+ significantly decreased by drought stress increasing. The highest value of LNH_4^+ was observed in D/P between four scion-rootstock combinations (Figure 3d).

3.8. Nitrogen uses efficiency traits

The results showed that drought stress levels and scion-rootstock compositions affected root and leaf nitrogen concentrations. LN concentrations in D/D and D/P were significantly higher than in L/D and L/P (Figure 1a). Moreover, high levels of drought stress decreased RN concentration which was 23.75 % lower in severe stress than in control (Figure 2d). $PNUE$ was significantly reduced under drought-stress conditions. Moderate and severe stress levels decreased $PNUE$ by about 40% and 50% as compared to the control (Figure 2b). TNA , $NUtE$, and $NUpE$ were significantly affected by the scion-rootstock combination and drought stress levels interactions (Figure 5). Drought stress significantly reduced TNA in L/D and D/D, but it was increased under moderate stress and then decreased by severe stress in L/P and D/P (Figure 5b). In contrast, drought stress levels had no significant effect on $NUpE$ and $NUtE$, and differences between scion-rootstocks were observed for them. The highest value of $NUtE$ was in D/P, and the $NUpE$ in L/D and D/D was higher than in L/P and D/P (Figures 5c and d).

4. Discussion

In the present study, severe drought stress significantly reduced TDW and root-to-shoot ratio (Figures 3a and b). Our results indicate that 'Dargazi' as cultivar had the highest amount of R/SH ratio and as rootstock showed more reduction for R/SH ratio under severe stress. 'Louise Bonne' cultivar on both rootstocks showed an increased R/SH ratio under moderate stresses (Figure 3b). It has been reported that water stress causes differences in the production of roots and root dry weight than shoot dry weight, which shows that plant species have different potential to develop roots that have more excellent resistance to water stress (Jaleel *et al.*, 2009). In some situations, drought caused a reduction in leaf morpho-physiological characterization like photosynthetic rate, chlorophyll concentrations, dry biomass, and nitrogen assimilation, which resulted in low carbohydrate production and poor nutrient metabolism and finally led to a reduction in growth (Huang *et al.*, 2018b). Our results showed that between all four combinations, D/D and D/P had the highest Chl concentration, indicating that the 'Dargazi' cultivar has more Chl concentration than 'Louise Bonne' (Figure 1b).

379 Osmotic adjustment, water-use efficiency, chlorophyll concentration, leaf water potential, leaf
 380 relative water content, growth capacity, transpiration rate, and photosynthetic ability are the main
 381 indicators of plant growth and water status, especially under drought conditions (Qian *et al.*, 2020).
 382 *An*, *PNUE*, *LRWC*, *SP*, and *EiC* were not different between the four scion-rootstock combinations,
 383 but they showed a decrease under the influence of drought stress (Figure 2a, b, c, g, and i). Rubisco
 384 and PEP carboxylase activity, known as major limiting factors for photosynthesis under drought
 385 stress conditions, as a result, causes a reduction in *EiC* and *An*, also there is a relationship between
 386 leaf *An* and *N* concentration as *PNUE* (Dinh *et al.*, 2017). Stomata are critical for regulating the
 387 uptake of CO₂ for photosynthesis and the evaporation of water for transpiration in plants which
 388 makes them important regulators of *WUE* (Aliniaiefard *et al.*, 2020; Flexas *et al.*, 2004). K^+ and
 389 Ca^{2+} have main roles as significant inorganic osmoticum in stomatal opening and closure, because
 390 stomatal closure occurs via Ca^{2+} channels and inhibition of the flow of K^+ ions into guard cells
 391 can be induced by ABA to control transpiration (Dayer *et al.*, 2020; Sussmilch *et al.*, 2017). We
 392 observed a positive correlation of g_s and *An* with LCa^{2+} and a negative correlation with LK^+ (Table
 393 5), which showed the role of K^+ and Ca^{2+} in stomatal conductance and photosynthesis rate by
 394 influencing stomata opening and closing. The *SL/SW* ratio and *WUEi* were increased and the *SA*
 395 decreased by increasing stress levels (Tables 4 and 5). D/D and D/P have the lowest *SA* value and
 396 subsequently, D/D showed the highest *WUEi*, in addition, L/P showed the most increased *SL/SW*
 397 ratio in comparing other scion-rootstock combinations in the present study. On the other side,
 398 stomata act as ports that control the evaporation of water for transpiration and the uptake of CO₂
 399 for photosynthesis. But, stomatal limitations are known as short-term responses to mild to
 400 moderate drought stress, whereas non-stomatal effects are usually considered to be more important
 401 during longer and more severe drought stress events (Roelfsema and Hedrich, 2002). However,
 402 *An*, *Ci*, and the *E* of the L/D and L/P leaves were significantly higher, indicating that the increase
 403 in the leaf photosynthetic efficiency in the 'Louise Bonne' cultivar may be a stomatal limiting factor
 404 (Wang *et al.*, 2018). Moreover, the aperture of stomata is a near-instantaneous, short-term response
 405 to environmental and endogenous signals, while the stomatal index and density are generally
 406 considered to be long-term developmental adaptations to the environment (Clavijo-Herrera *et al.*,
 407 2018). There was no significant difference in stomatal density between the four combinations
 408 under drought stress conditions in our study (Table 3). We find that the levels of RK^+ and LCa^{2+}
 409 were increased with increasing stress levels (Figures 6c and 2f), and the LK^+ and RCa^{2+} were

considerably decreased by increasing drought stress levels (Table 4 and Figure 6d). The amount of LK^+ content was lowest in L/P and D/P combinations, indicating stomatal closure in scions on 'Pyrodwarf' rootstock is more related to LK^+ induction than stomatal closure in scions on 'Dargazi' rootstock. In contrast, compatible solutes like *Prol*, *GB*, and *TSC* were increased under drought stress, and *Prol* was higher in D/D and D/P which had the highest stomatal closure (lowest g_s and *SA*) (Table 1), and there was a negative relation between *Prol* and *SA*, *An*, g_s , and LK^+ (Table 5). In addition, D/D and D/P showed the greatest decrease in osmotic potential under severe drought stress compared to control and moderate stress conditions (Figure 3c). These results showed stomatal movements in 'Dargazi' as a cultivar were more related to osmolytes than the 'Louise Bonne' cultivar. Our results agreed with those of authors who indicated a higher *Prol*, *GB*, and *TSC* accumulation under drought stress conditions, as a result, Ψ_s decreased which caused stomatal closure (Asayesh *et al.*, 2017). According to previous studies, *GB* is a mediator of osmoregulation and *Prol* is an important organic solute that plays a key role in the depot of energy to regulate redox potential. Also, *TSC* can produce and accumulate osmolites to avoid the negative effects of water stress, all known as the principal contributors to osmotic adjustment under osmotic stress (Blum, 2017). Photosynthesis is a varieties-specific dependent factor that influenced growth performance under stress. Besides, the concentration of soluble proteins may be used as an index of Rubisco protein, because the content of this protein in leaves includes 30–50% of the total soluble proteins (Sanchez-Rodriguez *et al.*, 2011), so drought stress also caused reductions in *PNUE* and *SP* in our study (Figures 2b and g). The positive relationship between *PNUE* and *An*, g_s , *EiC*, and *E*, indicates that with the decrease in the amount of gas exchange under drought stress conditions, *PNUE* is also affected and decreased (Table 5). In our study, a decrease in g_s and *E* for all scion-rootstock combinations was observed, D/D has the lowest value of g_s and *E* (Tables 2 and 4, Figure 5a). In addition, we find *Ci* decreased by drought level increasing (Table 4), and D/D and D/P showed lower values for *Ci* under drought stress (Table 2). These results confirmed the high capacity of 'Dargazi' as a cultivar or rootstock in conserving water loss under drought stress by decreasing g_s and *E* and increasing *WUEi*. Transpiration is the main reason for water loss in plants. Generally, under drought stress conditions, stomatal conductance and transpiration rate are significantly reduced, to maintain the leaf's relative water content (*LRWC*). In line with the current results, previous studies presented a significant reduction in growth traits and many physiological

processes such as *LRWC*, *Chl* index, photosynthesis, and other related traits under drought stress (Dinh *et al.*, 2017; Huang *et al.*, 2018a; Qian *et al.*, 2020; Xia *et al.*, 2020).

Earlier research revealed that drought conditions generally decrease the LK^+ content, without effect on the *N* and *P* contents in apple trees (Ferree and Schmid, 2000); however, drought stress decreases the *P*, *N*, and K^+ contents in leaves of *Malus hupehensis* (Liang *et al.*, 2017). In the current study, we found a decrease in *N* concentration by increasing drought stress in the root, but not in the shoot (Figures 1a and 2d). The *LN* concentration was different between scion-rootstock combinations, and it was more in D/D and D/P than in L/D and L/P (Figure 1a). The levels of *LP* and *RP* were increased with increasing stress levels and the *LP* content in L/P under moderate and severe stress levels showed a huge increase when compared to control plants (Figure 6b). That indicates 'Dargazi' as a cultivar and rootstock are efficient to *N* uptake more than 'Louise Bonne'. The positive correlation between *WUEi* and *LN* (Table 5) shows that under drought stress 'Dargazi' has high water and nitrogen accumulation in leaves. On the other hand, *TNA* had the highest decrease in D/D and D/P, especially under severe stress levels; also, L/P and D/P under moderate stress levels have the highest value of *TNA* (Figure 5b). These results indicate that the 'Dargazi' cultivar has a higher *N* accumulation in the leaf than in 'Louise Bonne'. Nevertheless, *TNA* increase under moderate stress showed that 'Pyrodwarf' rootstock has more ability to absorb and transfer *N*. *TNA* decreasing shows that the limitation of g_s and *E* under drought stress limits *N* uptake because *N* is necessary for regulating the adaptation of photosynthesis to water stress. Drought can affect nitrogen absorption and transport to the aboveground plant portions by decreasing the transpiration rate and membrane permeability (Iqbal *et al.*, 2020). We saw high NO_3^- concentration for D/D and D/P in comparison to L/D and L/P in the leaf. The roots NO_3^- under moderate stress for L/D, D/D, and L/P changed with an increasing trend, and then significantly decreased under severe stress conditions (Figures 4a and b). *NRA* in both leaf and root, apart from L/D that showed an increase under moderate stress, all four scion-rootstock combinations considerably decreased by stress level increasing (Figure 4c and d). The high NO_3^- in leaves and decrease of it in roots under severe stress levels may be due to high or low *NRA* in plants because there is a negative correlation between *NRA* and NO_3^- concentration and the activity state of *NR* is very dependent on water status (Abenavoli *et al.*, 2016). Under drought stress, the leaf, and root NH_4^+ showed a decreasing trend. RNH_4^+ in L/D and L/P was more than in D/D and D/P (Table 2), while LNH_4^+ had the highest value in D/P (Figure 3d). All of these results confirm that 'Dargazi' has high LNO_3^- and

LNH_4^+ and 'Louise Bonne' on both rootstocks had high LNH_4^+ and RNO_3^- , especially under moderate stress levels. Water deficit might modify *N* metabolism and allocation through the adjustment of enzymes involved in *N* assimilation. by reducing leaf area and *LRWC* under drought stress, shoot *N* demand can reduce, and that show, water deficit also affects *N* demand (Wang *et al.*, 2017). The result of several studies also indicated that drought stress considerably influenced *N* assimilation by reducing activities of key enzymes related to *N* assimilation (Huang *et al.*, 2018b; Iqbal *et al.*, 2020; Xia *et al.*, 2020). As main *NUE* parameters, we found *NUtE* and *NUpE* were different between four scion-rootstock combinations, by increasing drought stress levels, the highest *NUtE* was observed in D/P, and *NUpE* in L/D and D/D was more than in L/P and D/P (Figures 5c and d). According to these results, 'Dargazi' as a rootstock has a high efficiency of nitrogen absorption compared to 'Pyrodwarf' rootstock, especially in severe stress conditions. This can be the main reason for increasing *TNA* and *WUEi* and decreasing *g_s* and *E* in 'Dargazi' because of its deep and long roots as previously reported by Karbasi and Arzani (2021). Also as a cultivar, the efficiency of using nitrogen is high in 'Dargazi'. In general, *NUE* is a complex trait that can be dissected into plant physiological components, *NUpE* and *NUtE*. The *NUpE* defines the capacity of roots to accumulate *N* from the soil and it is associated with the activity of the NO_3^- uptake system, but the *NUtE* may be more dependent on *N* assimilation and storage in roots and shoots (Abenavoli *et al.*, 2016). Many previous studies confirmed that high *N* concentration as compared to low *N* concentration could improve growth, and there is a direct relationship between plant stress tolerance and *N* uptake and utilization (Abenavoli *et al.*, 2016;; Dinh *et al.*, 2017; Iqbal *et al.*, 2020).

5. Conclusion

The study concluded that the correct rootstock and scion-rootstock combinations are crucial for the growth of pears under drought-stress conditions. In particular, the 'Dargazi' seedling performed better than the 'Pyrodwarf' clonal rootstock under severe drought. In this study as mentioned, drought stress significantly influenced gas exchange and macronutrient efficiency. We observed that 'Dargazi' as a cultivar or rootstock, had higher potency under severe drought stress. In contrast, according to some gas exchange parameters, the 'Louise Bonne' cultivar had this response under moderate stress.

As a further check, we compared the macronutrient uptake, transfer, and *NUE* performance of all four scion-rootstock combinations under drought stress levels. 'Dargazi' cultivar had a higher concentration of *LN* than 'Louise Bonne', while the 'Pyrodwarf' rootstock absorbed more N and Ca^{2+} under moderate stress. Under drought stress, the highest value of LNO_3^- was observed in D/D and D/P, while the most RNO_3^- was found in L/D under moderate stress. The highest *NUtE* was in D/P, and *NUpE* in L/D and D/D was higher than in L/P and D/P, indicating that 'Dargazi' as a seedling rootstock has high efficiency of nitrogen absorption compared to 'Pyrodwarf' clonal rootstock, especially in severe stress conditions. As a cultivar, 'Dargazi' also had high nitrogen absorption efficiency compared to 'Louise Bonne'.

The study suggests that under moderate stress conditions, the 'Pyrodwarf' rootstock and 'Louise Bonne' cultivar have suitable tolerance, but when stress increases to a severe level, 'Dargazi' shows better resistance as rootstock and cultivar. These results may be due to the resistance of the 'Pyrodwarf' as the clonal with shallow roots and 'Dargazi' as the seedling rootstock with deep roots to moderate and severe water stress conditions, respectively. Although clonal rootstocks are commonly used for fruit crops, seedling rootstocks with deep roots may be more suitable under severe unfavorable growing conditions such as drought. Thus, further study on the proper selection of rootstocks and scion combinations is necessary for managing orchards under drought stress conditions for various fruit crops.

Acknowledgment

We would like to thank Tarbiat Modares University (TMU) for its financial support. This work was supported by Ph.D. Student Grant Program by TMU. In addition, greenhouse and laboratory facilities provided by Pomology Lab., Department of Horticultural Science at TMU are acknowledged.

References

Abenavoli, M.R., Longo, C., Lupini, A., Miller, A.J., Araniti, F., Mercati, F., Princi, M.P., Sunseri, F., 2016. Phenotyping two tomato genotypes with different nitrogen use efficiency. *Plant Physiology and Biochemistry* 107, 21-32.

Aliniaiefard, S., Asayesh, Z.M., Driver, J., Vahdati, K., 2020. Stomatal features and desiccation responses of Persian walnut leaf as caused by in vitro stimuli aimed at stomatal closure. *Trees* 34, 1219-1232.

Arzani K (1994) Horticultural and physiological aspects of vigor control in apricot (*Prunus armeniaca* L.) under orchard and controlled environment conditions. Ph.D. Thesis. Department of Plant Science, Massey University. New Zealand.

Arzani, K., 2017. The potential and limiting environmental conditions on fruit trees germplasm and yield of established orchards in Iran. In: Proceedings of the First International Horticultural Science Conference of Iran (IrHC2017). Tarbiat Modares University (TMU), Tehran Iran, p. 110. September 4-7 Abstracts Book.

Arzani, K., 2021. The National Asian Pear (*Pyrus serotina* Rehd) project in Iran: compatibility and commercial studies of introduced cultivars, *Acta Horticulturae*, pp. 91-98.

Asayesh, M.Z., Vahdati, K., Aliniaiefard, S., 2017. Investigation of physiological components involved in low water conservation capacity of in vitro walnut plants. *Scientia Horticulturae* 224, 1-7.

Asayesh, Z.M., Aliniaiefard, S., Vahdati, K., 2021. Stomatal morphology and desiccation response of Persian walnut tissue culture plantlets influenced by the gelling agent of in vitro culture medium. *Journal of Nuts* 12, 41-52.

Asayesh, Z.M., Arzani, K., Mokhtassi-Bidgoli, A., Abdollahi, H., 2022. Macronutrients Uptake and Nitrogen Use Efficiency in Dargazi Seedling and Pyrodwarf Clonal Rootstocks Under Drought Stress Conditions. *Seed and Plant Journal* 37 (4), 489-511 (In Persian)

Asayesh, Z.M., Arzani, K., Mokhtassi-Bidgoli, A., Abdollahi, H., 2023a. Enzymatic and non-enzymatic response of grafted and ungrafted young European pear (*Pyrus communis* L.) trees to drought stress. *Scientia Horticulturae* 310, 111745.

Asayesh, Z.M., Arzani, K., Mokhtassi-Bidgoli, A., Abdollahi, H., 2023b. Gas Exchanges and Physiological Responses Differ Among ‘Pyrodwarf’ Clonal and ‘Dargazi’ Seedling Pear (*Pyrus communis* L.) Rootstocks in Response to Drought Stress. *Journal of Soil Science and Plant Nutrition*, 1-16.

Babaei, L., Sharifani, M.M., Darvishzadeh, R., Abbaspour, N., Henareh, M., 2021. Impact of drought stress on photosynthetic response of some pear species. *International Journal of Horticultural Science and Technology* 8, 353-369.

561 Bates, L., Waldren, R., Teare, I., 1973. Rapid determination of free proline for water-stress
562 studies. *Plant and soil* 39, 205-207.

563 Black, B.L., Fuchigami, L.H., Coleman, G.D., 2002. Partitioning of nitrate assimilation among
564 leaves stems, and roots of poplar. *Tree Physiology* 22, 717-724.

565 Blum, A., 2017. Osmotic adjustment is a prime drought stress adaptive engine in support of
566 plant production. *Plant, cell & environment* 40, 4-10.

567 Bosa, K., Jadczyk-Tobjasz, E., Kalaji, M., Majewska, M., Allakhverdiev, S., 2014. Evaluating
568 the effect of rootstocks and potassium level on photosynthetic productivity and yield of pear trees.
569 *Russian journal of plant physiology* 61, 231-237.

570 Bradford, M., 1976. Rapid and sensitive method for the quantitation of microgram quantities
571 of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72, 248-254.

572 Brautigam, A., Gagneul, D., Weber, A.P., 2007. High-throughput colorimetric method for the
573 parallel assay of glyoxylic acid and ammonium in a single extract. *Analytical Biochemistry* 362,
574 151-153.

575 Clavijo-Herrera, J., Van Santen, E., Gomez, C., 2018. Growth, water-use efficiency, stomatal
576 conductance, and nitrogen uptake of two lettuce cultivars grown under different percentages of
577 blue and red light. *Horticulturae* 4, 16.

578 Dayer, S., Scharwies, J.D., Ramesh, S.A., Sullivan, W., Doerflinger, F.C., Pagay, V., Tyerman,
579 S.D., 2020. Comparing hydraulics between two grapevine cultivars reveals differences in stomatal
580 regulation under water stress and exogenous ABA applications. *Frontiers in plant science* 11, 705.

581 de Freitas, S.T., Mitcham, E.I., 2012. 3 factors involved in fruit calcium deficiency disorders.
582 *Horticultural Reviews* 40, 107-146.

583 Dinh, T.H., Watanabe, K., Takaragawa, H., Nakabaru, M., Kawamitsu, Y., 2017.
584 Photosynthetic response and nitrogen use efficiency of sugarcane under drought stress conditions
585 with different nitrogen application levels. *Plant Production Science* 20, 412-422.

586 Ferree, D.C., Schmid, J., 2000. Chemical thinning 'Fuji' apple in the midwest. *Journal of*
587 *American Pomological Society* 54, 61-67.

588 Ferrer, F., Pla, I., Fonseca, F., Dalurzo, H., Villar, J., 2008. Combining field and laboratory
589 methods to calculate soil water content at field capacity and permanent wilting point, 10th
590 Congress of the European Society for Agronomy. Bologna-Italy *Rivista di Agronomia*. 3: 279-
591 280.

592 Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T., 2004. Diffusive and metabolic
 593 limitations to photosynthesis under drought and salinity in C3 plants. *Plant biology* 6, 269-279.

594 Grieve, C., Grattan, S., 1983. Rapid assay for determination of water-soluble quaternary
 595 ammonium compounds. *Plant and soil* 70, 303-307.

596 Huang, L., Li, M., Shao, Y., Sun, T., Li, C., Ma, F., 2018a. Ammonium uptake increases in
 597 response to PEG-induced drought stress in *Malus hupehensis* Rehd. *Environmental and*
 598 *Experimental Botany* 151, 32-42.

599 Huang, L., Li, M., Zhou, K., Sun, T., Hu, L., Li, C., Ma, F., 2018b. Uptake and metabolism of
 600 ammonium and nitrate in response to drought stress in *Malus prunifolia*. *Plant Physiology and*
 601 *Biochemistry* 127, 185-193.

602 Ikinci, A., Bolat, I., Ercisli, S., Kodad, O., 2014. Influence of rootstocks on growth, yield, fruit
 603 quality and leaf mineral element contents of pear cv.'Santa Maria'in semi-arid conditions.
 604 *Biological Research* 47, 1-8.

605 Iqbal, A., Dong, Q., Wang, X., Gui, H., Zhang, H., Zhang, X., Song, M., 2020. High nitrogen
 606 enhance drought tolerance in cotton through antioxidant enzymatic activities, nitrogen
 607 metabolism, and osmotic adjustment. *Plants* 9, 178.

608 Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R.,
 609 Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and
 610 pigments composition. *Int. J. Agric. Biol* 11, 100-105.

611 Karbasi, M., and Arzani, K., (2021) Asian and European pear scion-rootstock interactions and
 612 Pyrodwarf rootstock root growth and distribution. *Acta Hortic.* 1315: 63-68.

613 Liang, B., Li, C., Ma, C., Wei, Z., Wang, Q., Huang, D., Chen, Q., Li, C., Ma, F., 2017.
 614 Dopamine alleviates nutrient deficiency-induced stress in *Malus hupehensis*. *Plant Physiology and*
 615 *Biochemistry* 119, 346-359.

616 Li, S.-X., Wang, Z.-H., Stewart, B., 2013. Responses of crop plants to ammonium and nitrate
 617 N. *Advances in agronomy* 118, 205-397.

618 Martinez, J.-P., Lutts, S., Schanck, A., Bajji, M., Kinet, J.-M., 2004. Is osmotic adjustment
 619 required for water stress resistance in the Mediterranean shrub *Atriplex halimus* L? *Journal of*
 620 *Plant Physiology* 161, 1041-1051.

Martinez, H.E., de Souza, B.P., Caixeta, E.T., de Carvalho, F.P., Clemente, J.M., 2020. Water deficit changes nitrate uptake and expression of some nitrogen related genes in coffee-plants (*Coffea arabica* L.). *Scientia Horticulturae* 267, 109254.

Qian, W., LIU, C.-h., HUANG, D., DONG, Q.-l., LI, P.-m., van NOCKER, S., 2020. Physiological evaluation of nitrogen use efficiency of different apple cultivars under various nitrogen and water supply conditions. *Journal of Integrative Agriculture* 19, 709-720.

Roelfsema, M.R.G., Hedrich, R., 2002. Studying guard cells in the intact plant: modulation of stomatal movement by apoplastic factors. *New Phytologist* 153, 425-431.

Sanchez-Rodriguez, E., Leyva, R., Constan-Aguilar, C., Romero, L., Ruiz, J., 2014. How does grafting affect the ionome of cherry tomato plants under water stress? *Soil Science and Plant Nutrition* 60, 145-155.

Seki, T., Kawashima, K., Shibata, K., Inoue, H., Umekiya, Y., 2007. Translocation of nutrients in Japanese pear 'Kosui' grafted by the 'Tree Joint' method, *Acta Horticulturae* 800, pp. 273-280.

Singh, J., 1988. A rapid method for determination of nitrate in soil and plant extracts. *Plant and soil* 110, 137-139.

Sussmilch, F.C., Brodribb, T.J., McAdam, S.A., 2017. What are the evolutionary origins of stomatal responses to abscisic acid in land plants? *Journal of Integrative Plant Biology* 59, 240-260.

Vandoorne, B., Mathieu, A.-S., Van den Ende, W., Vergauwen, R., Périlleux, C., Javaux, M., Lutts, S., 2012. Water stress drastically reduces root growth and inulin yield in *Cichorium intybus* (var. *sativum*) independently of photosynthesis. *Journal of Experimental Botany* 63, 4359-4373.

Wang, H., Yang, Z., Yu, Y., Chen, S., He, Z., Wang, Y., Jiang, L., Wang, G., Yang, C., Liu, B., 2017. Drought enhances nitrogen uptake and assimilation in maize roots. *Agronomy Journal* 109, 39-46.

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H., Mei, L., 2018. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. *Biology Open* 7 bio035279.

Wei-Feng, W., Yu-Zheng, Z., 2014. Water and nitrogen-use efficiencies of sweet sorghum seedlings are improved under water stress. *International Journal of Agriculture and Biology* 16(2):285-292.

Wu, F., Bao, W., Li, F., Wu, N., 2008. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. *Photosynthetica* 46, 40-48.

Xia, H., Xu, T., Zhang, J., Shen, K., Li, Z., Liu, J., 2020. Drought-induced responses of nitrogen metabolism in *Ipomoea batatas*. *Plants* 9, 1341.

Table 1. Stomatal aperture (*SA*), stomatal length/width ratio (*SL/SW* ratio), stomatal conductance (*g_s*), and proline concentration (*Prol*) of four different pear scion-rootstock combinations.

Scion-rootstock combinations	<i>SA</i> (μm)	<i>SL/SW</i> ratio	<i>g_s</i> ($\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	<i>Prol</i> ($\mu\text{mol g}^{-1}\text{FW}$)
L/D	1.50±0.12 ^a	1.43±0.02 ^b	0.29±0.02 ^a	32.09±1.3 ^{ab}
D/D	1.28±0.08 ^{bc}	1.42±0.06 ^b	0.2±0.04 ^b	35.19±1.5 ^a
L/P	1.45±0.09 ^{ab}	1.55±0.02 ^a	0.28±0.05 ^a	31.35±1.2 ^b
D/P	1.16±0.04 ^c	1.48±0.02 ^{ab}	0.27±0.04 ^a	35.38±1.6 ^a
<i>Two-way ANOVA analyses</i>				
<i>a</i>	0.292 ^{**}	0.04 [*]	0.020 ^{**}	38.92 [*]
<i>b</i>	1.033 ^{**}	0.084 ^{**}	0.398 ^{**}	171.84 ^{**}
<i>a×b</i>	0.078 ^{ns}	0.023 ^{ns}	0.006 ^{ns}	7.06 ^{ns}

Values represent the mean ± standard errors (n=4). Different letters in the same column show statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. a: scion-rootstock combinations, b: drought treatments, and a×b: their interactions at the end of the experiment. ns, *, and **: not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2. Intracellular CO_2 concentration (*C_i*), instantaneous water use efficiency (*WUE_i*), leaf K^+ , and root NH_4^+ (*RNH₄⁺*) of four different pear scion-rootstock combinations.

Scion-rootstock combinations	<i>C_i</i> ($\mu\text{mol CO}_2\text{ mol}^{-1}$)	<i>WUE_i</i> ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	Leaf K^+ (%)	<i>RNH₄⁺</i> ($\text{mg g}^{-1}\text{DW}$)
L/D	238.3±4.12 ^a	7.3±0.4 ^c	1.98±0.15 ^a	19.7±0.2 ^a
D/D	224.7±2.92 ^b	10.54±0.7 ^a	1.73±0.1 ^b	18.1±0.3 ^b
L/P	234.09±3.9 ^{ab}	7.89±0.7 ^{bc}	1.52±0.08 ^b	19.3±0.1 ^a
D/P	225.6±3.3 ^b	9.21±0.6 ^{ab}	1.63±0.07 ^b	17.8±0.2 ^b
<i>Two-way ANOVA analyses</i>				
<i>a</i>	522.76 [*]	25.05 ^{**}	0.35 ^{**}	0.079 ^{**}
<i>b</i>	696.40 [*]	35.28 ^{**}	0.63 ^{**}	0.035 ^{**}

674 $a \times b$ 118.62^{ns} 3.33^{ns} 0.11^{ns} 0.006^{ns}
675 Values represent the mean \pm standard errors (n=4). Different letters in the same column show statistically significant
676 differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. a: scion-rootstock combinations, b:
677 drought treatments, and $a \times b$: their interactions at the end of the experiment. ns, *, and **: not significant, significant
678 at $P \leq 0.05$ and $P \leq 0.01$, respectively.

679 **Table 3.** Effect of three levels of drought stress (Control, Moderate, and Severe) on stomatal
680 aperture (*SA*), stomatal length/width ratio (*SL/SW* ratio), stomatal conductance (*g_s*), and proline
681 concentration (*Prol*) of four different pear scion-rootstock combinations.

Drought level	<i>SA</i> (μm)	<i>SL/SW</i> ratio	Stomatal density (no. mm^{-2})	<i>g_s</i> ($\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	<i>Prol</i> ($\mu\text{mol g}^{-1}\text{FW}$)
Control	1.58 \pm 0.08 ^a	1.41 \pm 0.01 ^b	186.6 \pm 8.2	0.44 \pm 0.02 ^a	29.65 \pm 0.7 ^c
Moderate	1.39 \pm 0.07 ^b	1.44 \pm 0.04 ^b	169.8 \pm 9.6	0.2 \pm 0.01 ^b	33.64 \pm 1.03 ^b
Severe	1.08 \pm 0.03 ^c	1.55 \pm 0.01 ^a	162.9 \pm 5.6	0.14 \pm 0.01 ^c	37.22 \pm 1.7 ^a
<i>Two-way ANOVA analyses</i>					
<i>a</i>	0.292 ^{**}	0.04 [*]	1153.98 ^{ns}	0.020 ^{**}	38.92 [*]
<i>b</i>	1.033 ^{**}	0.084 ^{**}	2370.04 ^{ns}	0.398 ^{**}	171.84 ^{**}
<i>a</i> \times <i>b</i>	0.078 ^{ns}	0.023 ^{ns}	296.73 ^{ns}	0.006 ^{ns}	7.06 ^{ns}

682 Values represent the mean \pm standard errors (n=4). Different letters in the same column show statistically significant
683 differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. a: scion-rootstock combinations, b:
684 drought treatments, and $a \times b$: their interactions at the end of the experiment. ns, *, and **: not significant, significant
685 at $P \leq 0.05$ and $P \leq 0.01$, respectively.
686

687 **Table 4.** Effect of three levels of drought stress (Control, Moderate, and Severe) on), intracellular
688 CO₂ concentration (*C_i*), instantaneous water use efficiency (*WUE_i*), leaf K⁺ (*LK⁺*), and root NH₄⁺
689 (*RNH₄⁺*) of four different pear scion-rootstock combinations.

Drought level	<i>C_i</i> ($\mu\text{mol CO}_2\text{ mol}^{-1}$)	<i>WUE_i</i> ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	<i>LK⁺</i> (%)	<i>RNH₄⁺</i> ($\text{mg g}^{-1}\text{DW}$)
Control	237.8 \pm 1.5 ^a	7.22 \pm 0.2 ^c	1.96 \pm 0.11 ^a	19.2 \pm 0.2 ^a
Moderate	229.5 \pm 3.1 ^{ab}	8.8 \pm 0.6 ^b	1.68 \pm 0.07 ^b	18.8 \pm 0.2 ^a
Severe	224.8 \pm 4.06 ^b	10.19 \pm 0.7 ^a	1.51 \pm 0.07 ^b	18.2 \pm 0.3 ^b
<i>Two-way ANOVA analyses</i>				
<i>a</i>	522.76 [*]	25.05 ^{**}	0.35 ^{**}	0.079 ^{**}
<i>b</i>	696.40 [*]	35.28 ^{**}	0.63 ^{**}	0.035 ^{**}
<i>a</i> \times <i>b</i>	118.62 ^{ns}	3.33 ^{ns}	0.11 ^{ns}	0.006 ^{ns}

690 Values represent the mean \pm standard errors (n=4). Different letters in the same column show statistically significant
691 differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. a: scion-rootstock combinations, b:
692 drought treatments, and $a \times b$: their interactions at the end of the experiment. ns, and **: not significant, and significant
693 at $P \leq 0.01$, respectively.
694

Table 5. Pearson’s correlation analysis of some studied parameters of four different scion-rootstock combinations.

	<i>An</i>	<i>gs</i>	<i>Ci</i>	<i>E</i>	<i>WUEi</i>	<i>EiC</i>	<i>PNUE</i>	<i>SA</i>	<i>Prol</i>	<i>GB</i>	<i>TSC</i>	<i>SP</i>	<i>LK</i> ⁺	<i>LCa</i> ²⁺	<i>LNO</i> ₃ ⁻	<i>LNH</i> ₄ ⁺	<i>LNRA</i>	<i>RN</i>	<i>LN</i>	<i>TNA</i>	
<i>An</i>	1																				
<i>gs</i>	**	1																			
<i>Ci</i>	*	**	1																		
<i>E</i>	**	**	**	1																	
<i>WUEi</i>	*	**	**	**	1																
<i>EiC</i>	**	**		**	*	1															
<i>PNUE</i>	**	**	**	**	*	**	1														
<i>SA</i>	*	*		*				1													
<i>Prol</i>	**	**	**	**	**	**	**	*	1												
<i>GB</i>	**	**	*	**		**	**		*	1											
<i>TSC</i>	*	*		*		*	*		**		1										
<i>SP</i>	**	**		**	*	**	**	*	**	**	**	1									
<i>LK</i> ⁺	*	*		*		*	*		*		**		1								
<i>LCa</i> ²⁺	**	**		**		**	**	**	*	**	**	**	*	1							
<i>LNO</i> ₃ ⁻			**		**				**			*			1						
<i>LNH</i> ₄ ⁺	*															1					
<i>LNRA</i>																**	1				
<i>RN</i>	*	*		*		*	*		**		**	**	**	**				1			
<i>LN</i>			**		**		*		**			*			**				1		
<i>TNA</i>						*				*		*		*						1	
	-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

696 The squares that received the white, bold blue, and red colors belong to the category of non-significant correlative values, and positive and negative correlative
697 values respectively. *: Significant at $P \leq 0.05$, **: Significant at $P \leq 0.01$. *A_n*: the net photosynthetic rate, *g_s*: stomatal conductance, *C_i*: intracellular CO₂
698 concentration, *E*: transpiration rate, *WUE_i*: instantaneous water use efficiency, *E_{iC}*: instantaneous carboxylation efficiency of Rubisco, *PNUE*: photosynthetic
699 nitrogen-use efficiency, *SA*: stomatal aperture, *Prol*: proline, *GB*: glycine betaine, *TSC*: total soluble carbohydrates, *SP*: soluble protein, *LK*⁺: leaf potassium,
700 *LCa*²⁺: leaf calcium, *LNO*₃⁻: leaf nitrate, *LNH*₄⁺: leaf ammonium, *LNRA*: leaf nitrate reductase activity, *RN*: root nitrogen concentration, *LN*: leaf nitrogen
701 concentration, *TNA*: total nitrogen accumulation.

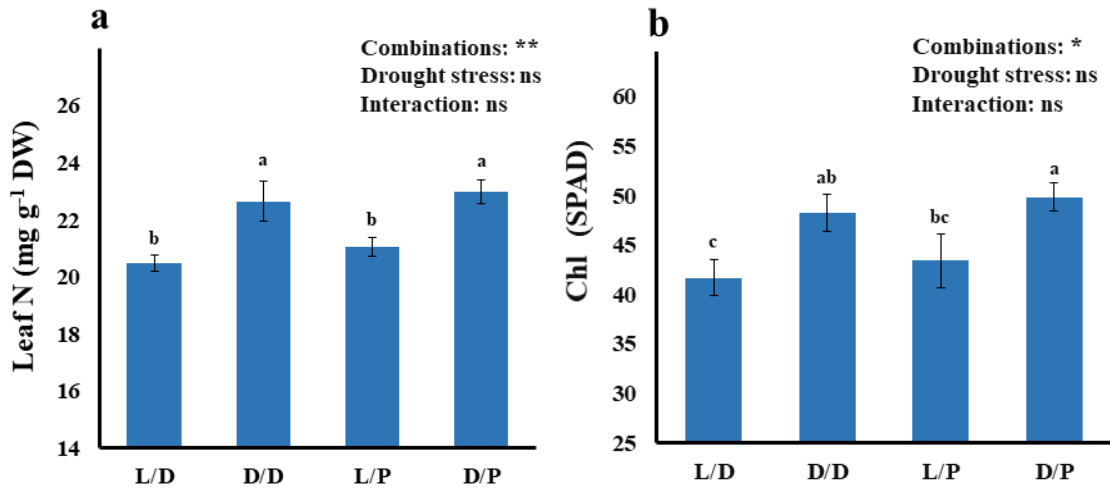


Figure 1. Leaf nitrogen concentration (a), and chlorophyll concentration (b) of four different pear scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf'). Vertical bars represent the \pm standard error of the mean (n=4). Different letters above individual bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, *, and **: not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

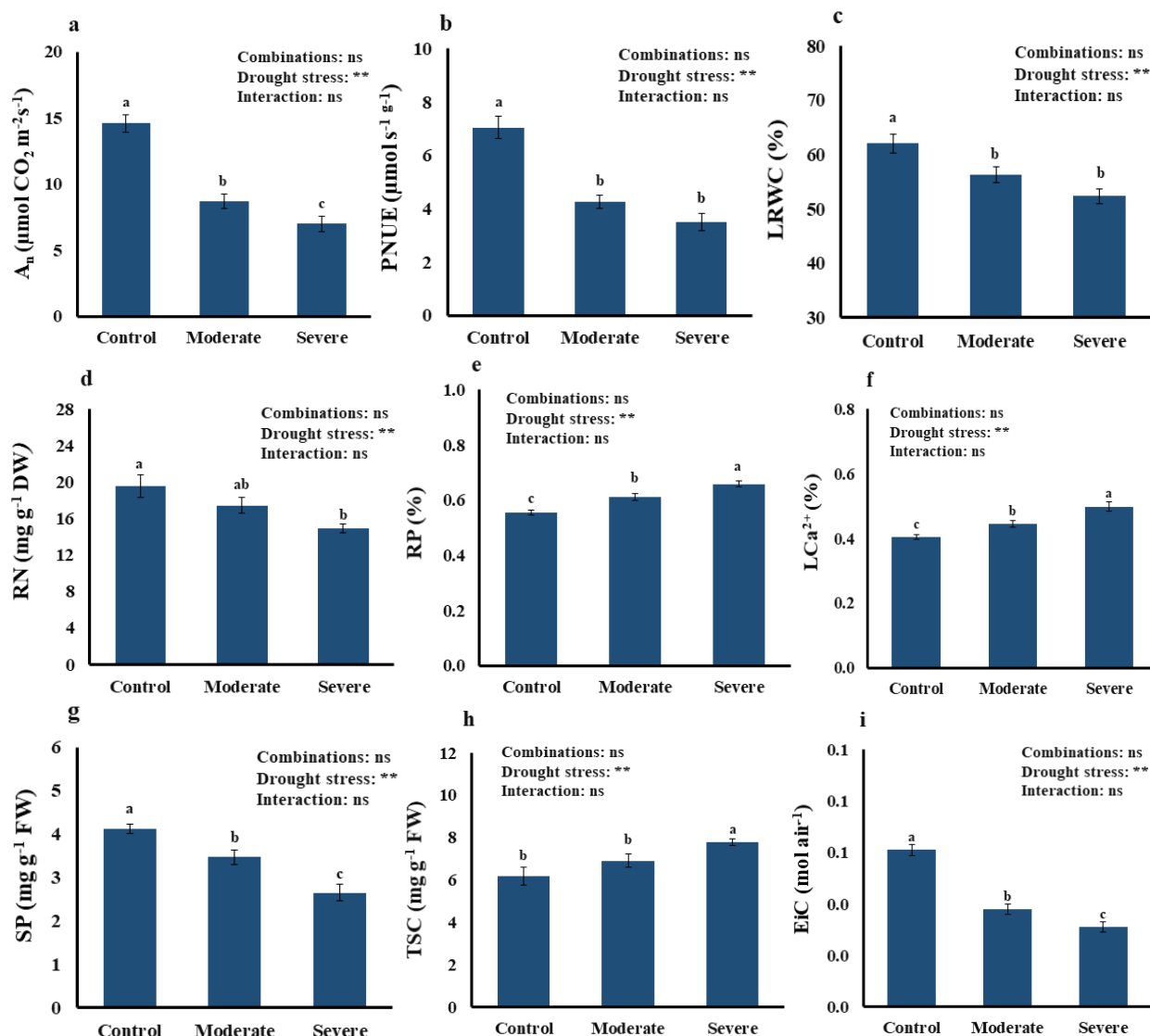


Figure 2. Effect of three levels of drought stress (Control, Moderate, and Severe) on net photosynthetic rate (a), photosynthetic nitrogen use efficiency (b), leaf relative water content (c), root nitrogen concentration (d), root phosphorus content (e), leaf calcium content (f), soluble protein (g), total soluble carbohydrates(h), and instantaneous carboxylation efficiency of Rubisco (i) of four different pear scion-rootstock combinations. Vertical bars represent the \pm standard error of the mean ($n=4$). Different letters above individual bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, and **: not significant, and significant at $P \leq 0.01$, respectively.

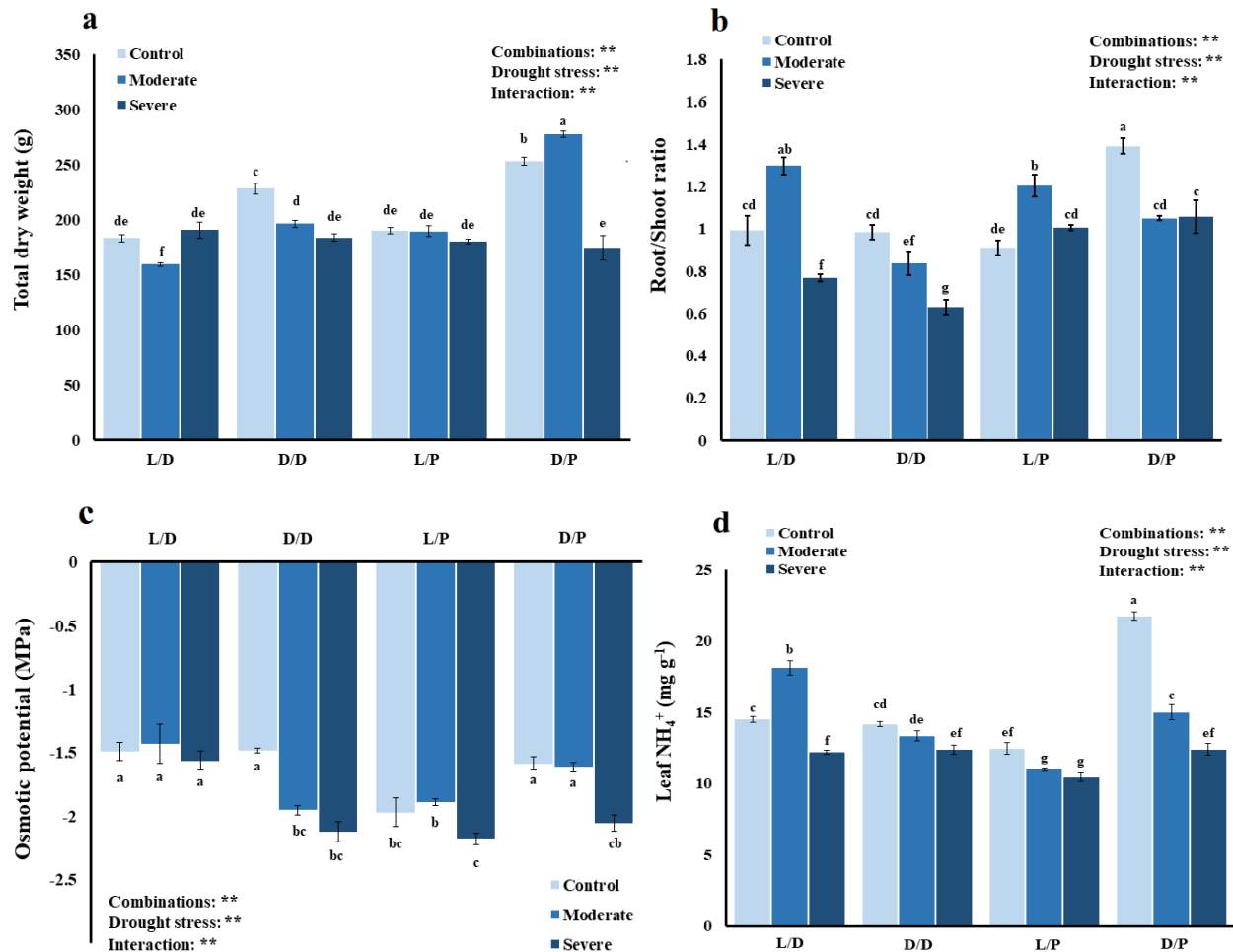


Figure 3. a: total dry weight (*TDW*), b: root-to-shoot ratio, c: osmotic potential (Ψ_s), d: leaf NH_4^+ of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different drought stress conditions (Control, Moderate, Severe). Vertical bars represent the \pm standard error of the mean ($n=4$). Different letters above individual bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, and **: not significant, and significant at $P \leq 0.01$, respectively.

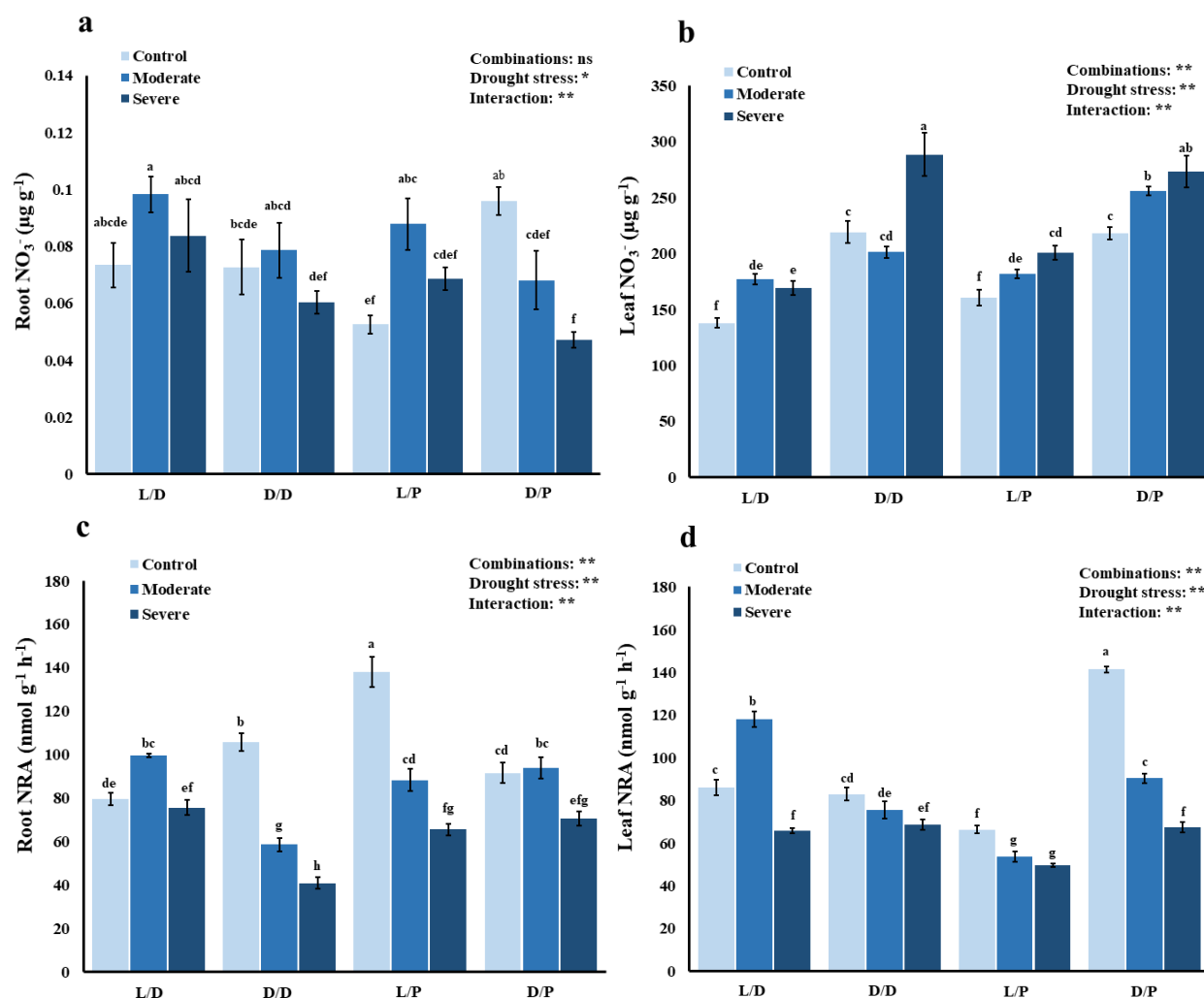


Figure 4. a: root NO_3^- , b: leaf NO_3^- , c: root nitrate reductase activity (NRA), d: leaf nitrate reductase activity (NRA) of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different drought stress conditions (Control, Moderate, Severe). Vertical bars represent the \pm standard error of the mean (n=4). Different letters above individual bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, *, and **: not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

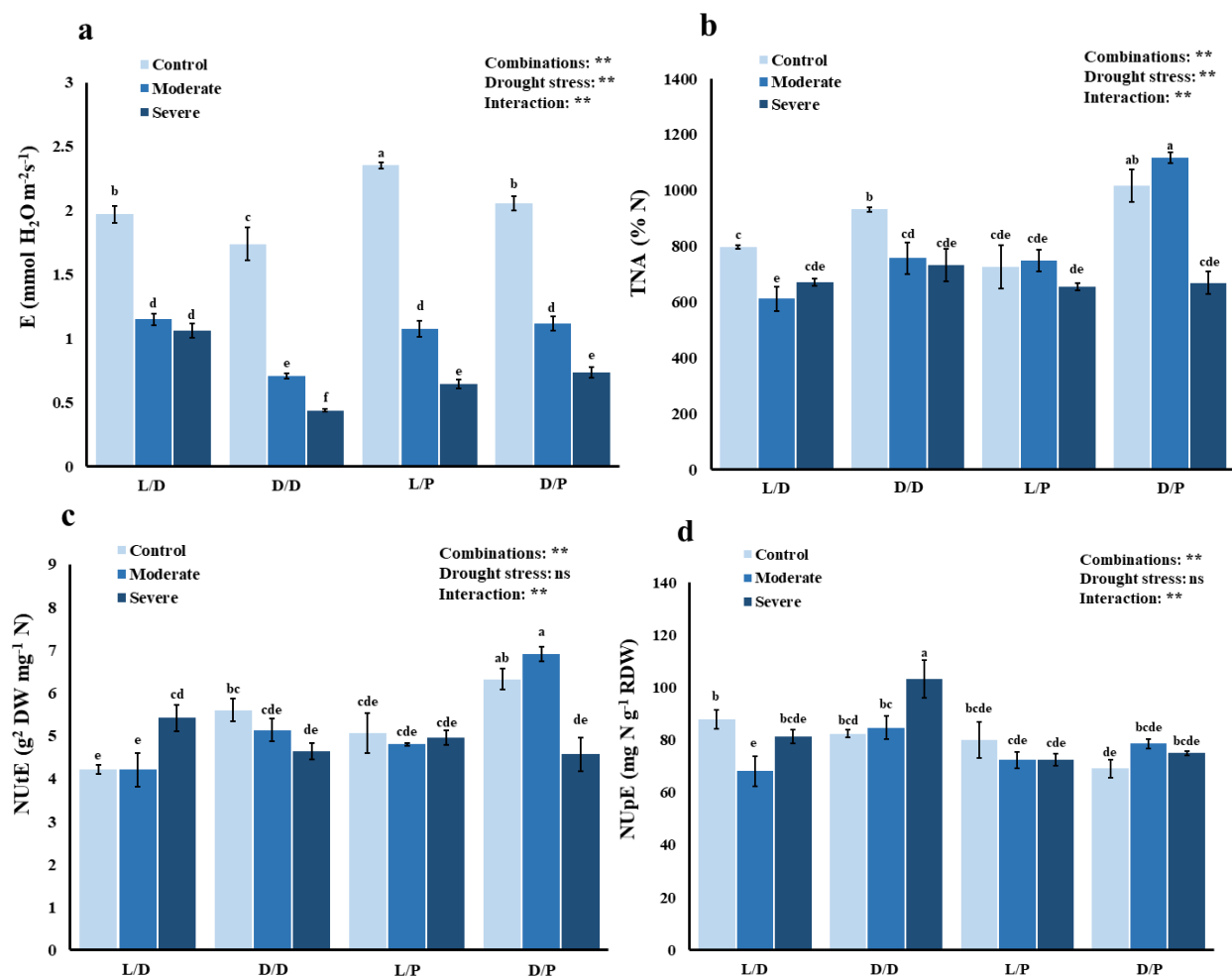


Figure 5. a: transpiration rate (E), b: total nitrogen accumulation (TNA), c: nitrogen utilization efficiency (NUE), d: nitrogen uptake efficiency ($NUpE$) of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different drought stress conditions (Control, Moderate, Severe). Vertical bars represent the \pm standard error of the mean ($n=4$). Different letters above individual bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, and **: not significant, and significant at $P \leq 0.01$, respectively.

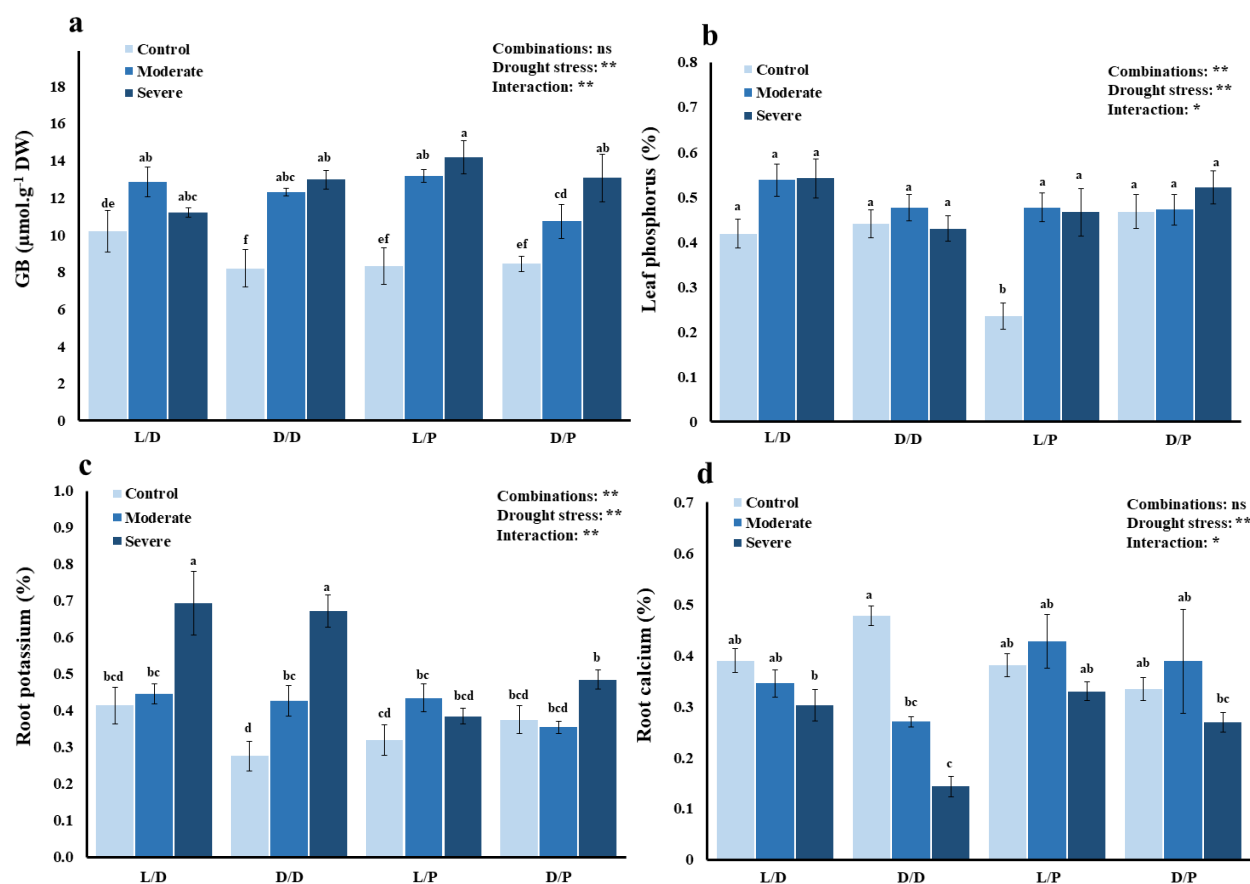


Figure 6. a: glycine betaine (*GB*), b: leaf phosphorus (*LP*), c: root potassium (*RK*⁺), d: root calcium (*RCa*²⁺) of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different drought stress conditions (Control, Moderate, Severe). Vertical bars represent the \pm standard error of the mean (n=4). Different letters above individual bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, *, and **: not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.