

Drought Stress Effects on the Aerial Part Physiology and Macronutrient Uptake in Pear (*Pyrus communis* L.) Scion-Rootstock Combinations

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ABSTRACT

Drought is a major abiotic stress in arid and semi-arid areas. This study examines how drought stress and different rootstocks affect aerial part physiology and macronutrient uptake in two seedlings of pear cultivars, 'Dargazi' and 'Louise Bonne,' grafted onto 'Dargazi' and 'Pyrodwarf' rootstocks. Treatments were well-watered as control (100% Field Capacity, FC), moderate (60% FC), and severe drought (30% FC). Drought stress decreased the Root-to-Shoot weight ratio (R/SH), 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), net photosynthetic rate (An), transpiration rate (E), stomatal conductance (gs), and instantaneous carboxylation Efficiency of Rubisco (EiC) in all scion-rootstock combinations. Under drought stress, levels of proline (Prol), Glycine Betaine (GB), Total Soluble Carbohydrate (TSC), root potassium (RK^+), leaf calcium (LCa^{2+}), leaf nitrogen (LN), leaf and root phosphorus (P) increased. 'Dargazi' as the cultivar or rootstock showed better instantaneous Water Use Efficiency (WUEi), Nitrogen Utilization Efficiency (NUE), and Nitrogen Uptake Efficiency (NUE), with higher LN, LNO^{3-} , LNH^{4+} , Prol, and Ψ_s under severe stress. 'Louise Bonne' had higher amounts of R/SH weight ratio, Total Nitrogen Accumulation (TNA), leaf and root nitrate reductase activity (NRA), An, intracellular carbon dioxide concentration (Ci), E, and root nitrate (RNO^{3-}) under moderate stress. 'Pyrodwarf' rootstock demonstrated the highest TNA and RCa^{2+} under moderate stress conditions. These results indicated that 'Dargazi' demonstrated better tolerance under severe stress, while 'Pyrodwarf' and 'Louise Bonne' were more effective under moderate stress conditions.

Keywords: Abiotic stresses, Osmolytes, Photosynthesis, Scion-rootstock interaction.

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-

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Rodriguez *et al.*, 2011). 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, 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 changes in plant's water status 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 lead 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 grows 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 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 that 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; İkinci *et al.*, 2014). Trees vigor enhancement by the root system of rootstock and its effect on plant yield could increase water and nutrient uptake in grafted plants. The mineral content 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.*, 2011; Seki *et al.*, 2007). Although cultivation of fruit trees is being shifted to the clonal rootstocks in most fruit-growing countries, because of their deep root, seedling rootstocks might be more suitable 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, were reported for nutrient uptake efficiency 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. This study aimed to enhance our understanding of the relationship between stomatal movement and gas exchange in the aerial parts of pear plants, particularly under drought stress. We investigated four combinations of pear scions and rootstocks, grafted onto two distinct ones. Our goal was to assess how these factors influence micronutrient uptake and utilization. Additionally, the study aimed to clarify the effectiveness of rootstocks on the nutrient status of the plants to optimize fertilization programs and

improve the management of pear orchards under water stress conditions.

MATERIALS AND METHODS

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.

Drought Treatment Application

The experimental trees were well-watered and allowed to grow for four months in the greenhouse. 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. 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).

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 weight 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) and Wu *et al.* (2008) as the following formula:

$$\text{LRWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

Where, FW is the Fresh Weight, TW is the Turgid Weight after rehydrating samples for 24 hours, and DW is the weight recorded after oven-drying samples at 85°C for 24 hours.

Leaf Osmotic Potential 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 (Bates *et al.*, 1973). The absorbance of free proline content ($\mu\text{g g}^{-1}$ FW) was read at 520 nm, and calculated based on a standard curve prepared with five standard concentrations (0–200 $\mu\text{g mL}^{-1}$) of L-proline.

Glycine Betaine (GB) content ($\mu\text{g g}^{-1}$ DW) 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 $\mu\text{g mL}^{-1}$ glycine betaine as prepared in 2M sulfuric.

Total soluble carbohydrates and Soluble Protein

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 the reference standards of Bovine Serum Albumin (BSA).

Stomatal Morphology

To examine stomatal differences in pear scion-rootstock combinations, we analyzed the stomata of the lower epidermis on fully expanded leaves from 4 randomly selected leaves. A total of 80 stomata from each leaf were studied using an Olympus BX51 light microscope, following the methodology of 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.

Gas Exchange Measurements

Photosynthesis and gas exchange include net photosynthetic rate (A_n), transpiration rate (E), stomatal conductance (g_s), and intracellular CO_2 concentration (C_i) and 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 was 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 (WUEi) and instantaneous carboxylation Efficiency of Rubisco (EiC), were calculated as the ratio between net Photosynthesis and transpiration rate ($\text{WUEi} = A_n/E$) and the ratio between net photosynthesis and intracellular CO_2 concentration ($\text{EiC} = A_n/C_i$).

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 2N HCL, was used. This extract was 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.

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). One 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 (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 μL chloroform), the extraction solution was shaken for 15 min and centrifuged at $10,000\times g$ for 10 minutes at 4°C . The aqueous phase was transferred to a 2 mL tube with 50 mg of activated charcoal and centrifuged at $12,000\times g$, for 5 min at 4°C . Afterward, NH_4^+ concentration in the supernatant phase was measured at 620 nm.

Nitrate reductase activity (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 and 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 mL of 1% sulphanilamide in 3M HCl and 500 mL 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 was measured at 540 nm with a spectrophotometer (SmartSpec Plus Spectrophotometer, BIO-RAD). Finally, enzyme activity was calculated by comparing the amount of nitrite produced after 90-min incubation with that detected after 10 min (Black *et al.*, 2002). We took the mean of two replicates and expressed NRA as μmol nitrite produced in g^{-1} fresh-mass in the fine roots or leaves h^{-1} .

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 distilled with concentrated sulfuric acid and 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 (g TDW mg^{-1} N)
- Nitrogen Uptake Efficiency (NUPE)= TNA/Root Dry Weight (mg N g^{-1} 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

Statistical Analysis

This experiment was conducted as a factorial experiment based on the Randomized Complete Block Design (RCBD) with 4 scion-rootstock combinations, and 3 levels of drought stress



as 2 factors with 4 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 used 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.

RESULTS

Morpho-Physiologic Characteristics

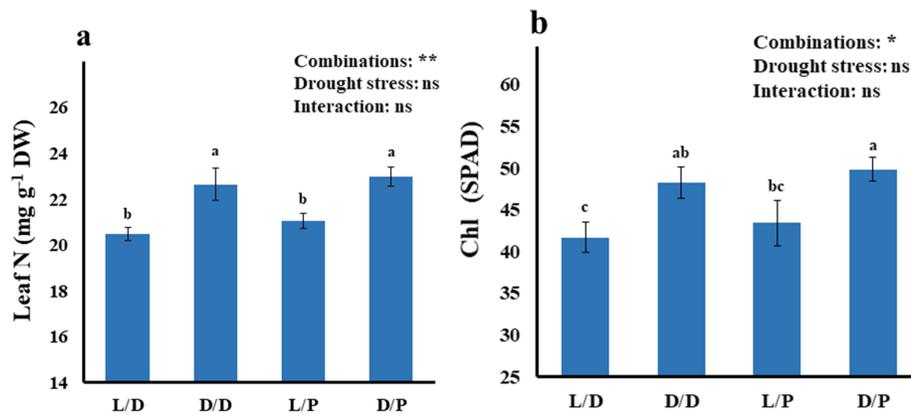


Figure 1. Leaf nitrogen concentration (a), and chlorophyll concentration (b) of four different pear scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf'). Vertical bars represent the \pm standard error of the 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.

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 the 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 the control (Figure 3-a). 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 3-b). 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 1-b), 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 the control conditions, respectively (Figure 2-c).

Stomatal Morphology

Leaf Osmotic Potential and Compatible Solutes

The Ψ_s was highly affected by scion-rootstock combinations and the interaction of scion-rootstock combinations and drought (Figure 3-c). We observed the lowest amount of Ψ_s in L/D among the 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 the control (Figure 3-c). 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).

Total Soluble Carbohydrate and Soluble Protein

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 2-h). 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 2-g).

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*, *gs*, *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).

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 2-a). 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 values 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

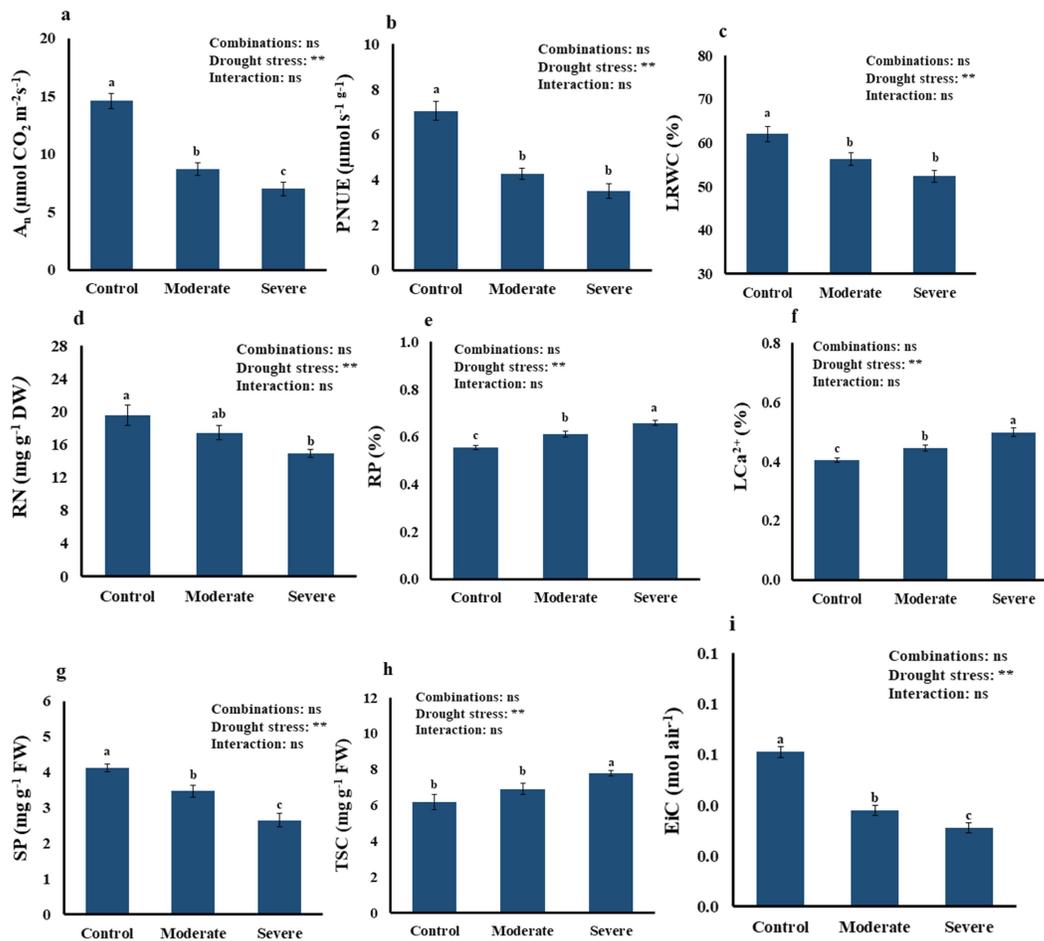


Figure 2. Effect of three levels of drought stress (control, moderate, and severe) on net photosynthetic rate (a), photosynthetic nitrogen use efficiency (b), leaf relative water content (c), root nitrogen concentration (d), root phosphorus content (e), leaf calcium content (f), soluble protein (g), 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.

compared to the control was about two times in L/D and D/P and three times in D/D and L/P (Figure 5-a). Our results showed a negative significant correlation between A_n , E, and g_s with WUE_i , Prol, GB, TSC, and LCa^{2+} and a positive relation with SP, LK^+ , and RN (Table 5).

WUE_i and EiC factors were significantly different between drought stress levels. WUE_i was increased (Table 4) and EiC was decreased (Figure 2-i) by increasing drought stress levels. WUE_i 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 the control conditions. We observed a negative correlation between the PNUE, EiC, and SP with WUE_i , in contrast, there were significant direct correlations between WUE_i with Prol, LNO_3^- , and LN (Table 5).

A substantial change in Ci value was observed for all different scion-rootstock combinations with increasing drought stress (Table 2), and a similar trend was observed

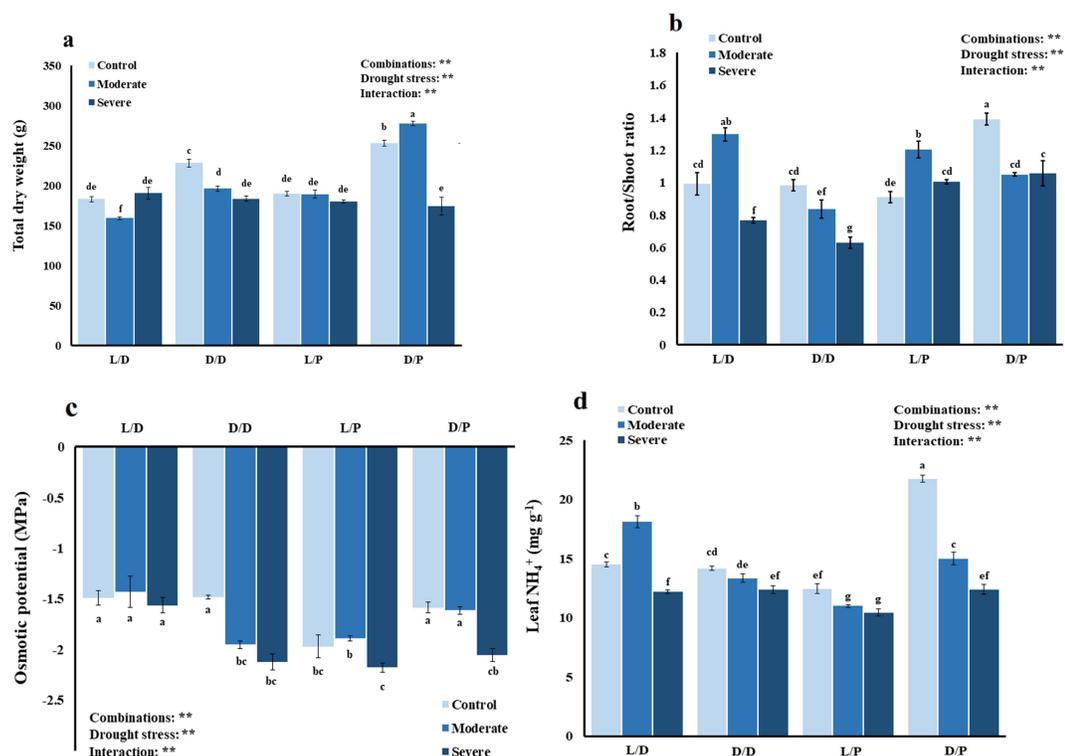


Figure 3. (a) Total Dry Weight (TDW), (b) Root-to-shoot ratio, (c) osmotic potential (Ψ_s), and (d) Leaf NH_4^+ of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different drought stress 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.

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.^a

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

^a Values represent the mean \pm 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**x**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 CO₂ concentration (Ci), instantaneous Water Use Efficiency (WUEi), leaf K⁺, and Root NH₄⁺ (RNH₄⁺) of four different pear scion-rootstock combinations.^a

Scion-rootstock combinations	Ci (μmol CO ₂ mol ⁻¹)	WUEi (μmol CO ₂ mmol ⁻¹ H ₂ O)	Leaf K ⁺ (%)	RNH ₄ ⁺ (mg g ⁻¹ 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
Two-way ANOVA analyses				
a	522.76 [*]	25.05 ^{**}	0.35 ^{**}	0.079 ^{**}
b	696.40 [*]	35.28 ^{**}	0.63 ^{**}	0.035 ^{**}
a×b	118.62 ^{ns}	3.33 ^{ns}	0.11 ^{ns}	0.006 ^{ns}

^a Values represent the mean±standard errors (n= 4). Different letters in the same column show statistically significant differences among treatments at P≤ 0.05 based on Duncan's multiple range test. Small letter symbols are defined under Table 1.

Table 3. Effect of three levels of drought stress (control, moderate, and severe) on 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.^a

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

^a Values represent the mean±standard errors (n= 4). Different letters in the same column show statistically significant differences among treatments at P≤ 0.05 based on Duncan's multiple range test. Small letter symbols are defined under Table 1.

Table 4. Effect of three levels of drought stress (control, moderate, and severe) on intracellular CO₂ concentration (Ci), instantaneous Water Use Efficiency (WUEi), Leaf K⁺ (LK⁺), and Root NH₄⁺ (RNH₄⁺) of four different pear scion-rootstock combinations.^a

Drought level	Ci (μmol CO ₂ mol ⁻¹)	WUEi (μmol CO ₂ mmol ⁻¹ H ₂ O)	LK ⁺ (%)	RNH ₄ ⁺ (mg g ⁻¹ DW)
Control	237.8±1.5 ^a	7.22±0.2 ^c	1.96±0.11 ^a	19.2±0.2 ^a
Moderate	229.5±3.1 ^{ab}	8.8±0.6 ^b	1.68±0.07 ^b	18.8±0.2 ^a
Severe	224.8±4.06 ^b	10.19±0.7 ^a	1.51±0.07 ^b	18.2±0.3 ^b
Two-way ANOVA analyses				
a	522.76 [*]	25.05 ^{**}	0.35 ^{**}	0.079 ^{**}
b	696.40 [*]	35.28 ^{**}	0.63 ^{**}	0.035 ^{**}
a×b	118.62 ^{ns}	3.33 ^{ns}	0.11 ^{ns}	0.006 ^{ns}

^a Values represent the mean±standard errors (n= 4). Different letters in the same column show statistically significant differences among treatments at P≤ 0.05 based on Duncan's multiple range test. Small letter symbols are defined under Table 1.

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 Ci (Table 2). On the other hand, with increasing drought stress levels, the amounts of Ci 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).

K⁺, P, Ca²⁺ Concentration

According to our results, the drought stress significantly influenced the macronutrient amount in the leaf and root. The levels of LP, RK⁺ (Figures 6-b and -c), RP, and LCa²⁺ (Figures 2-e 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 compared to the control plants. In contrast, the levels of LK⁺ and RCa²⁺ were considerably decreased by increasing drought stress levels (Table 2 and Figure 6-d). The RCa²⁺ 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 LCa²⁺, LNO₃⁻, TSC, LN, and Prol under drought stress conditions (Table 5).

NO₃⁻, NH₄⁺, and NRA Measurements

The NO₃⁻ and NRA in leaf and root were influenced by the scion-rootstock combinations and drought stress levels interactions. The LNO₃⁻ 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 4-b). In contrast, RNO₃⁻ under moderate stress for L/D, D/D, and L/P changed with an increasing trend, and all four scion-rootstock combinations

significantly decreased under severe stress conditions (Figure 4-a). 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 increase in stress level. 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 4-c and -d). The RNH₄⁺ 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 LNH₄⁺ significantly decreased by increase in drought stress. The highest value of LNH₄⁺ was observed in D/P between the four scion-rootstock combinations (Figure 3-d).

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 1-a). Moreover, high levels of drought stress decreased RN concentration, which was 23.75% lower in severe stress than in the control (Figure 2-d). 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 2-b). 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 increased under moderate stress and decreased by severe stress in L/P and D/P (Figure 5-b). In contrast, drought stress levels had no significant effect on NUpE and NUtE, and differences between scion-rootstocks were observed for them. The



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

	An	gs	Ci	E	WUEi	EiC	PNUE	SA	Prol	GB	TSC	SP	LK ⁺	LCa ²⁺	LNO ₃ ⁻	LNH ₄ ⁺	LNRA	RN	LN	TNA	
An	1																				
gs	**	1																			
Ci	*	**	1																		
E	**	**	**	1																	
WUEi	*	**	**	**	1																
EiC	**	**	**	**	*	1															
PNUE	**	**	**	**	*	**	1														
SA	*	*	*	*	*	*	*	1													
Prol	**	**	**	**	**	**	**	*	1												
GB	**	**	*	**	**	**	**	*	*	1											
TSC	*	*	*	*	*	*	*	*	**	*	1										
SP	**	**	*	**	*	**	**	*	**	**	**	1									
LK ⁺	*	*	*	*	*	*	*	*	*	*	*	*	1								
LCa ²⁺	**	**	*	**	**	**	**	*	*	*	**	*	*	1							
LNO ₃ ⁻	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1						
LNH ₄ ⁺	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1					
LNRA	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1				
RN	*	*	*	*	*	*	*	*	**	**	**	**	**	**	**	**	**	1			
LN	*	*	**	*	**	*	*	*	**	**	*	*	*	*	*	*	*	*	1		
TNA	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	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

^a 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 values respectively. *: Significant at $P \leq 0.05$, **: Significant at $P \leq 0.01$. An: The net photosynthetic rate, gs: Stomatal conductance, Ci: Intracellular CO₂ concentration, E: Transpiration rate, WUEi: Instantaneous Water Use Efficiency, EiC: Instantaneous Carboxylation Efficiency of Rubisco, PNUE: Photosynthetic Nitrogen-Use Efficiency, SA: Stomatal Aperture, Prol: Proline, GB: Glycine Betaine, TSC: Total Soluble Carbohydrates, SP: Soluble Protein, LK⁺: Leaf potassium, LCa²⁺: Leaf Calcium, LNO₃⁻: Leaf Nitrate, LNH₄⁺: Leaf Ammonium, LNRA: Leaf Nitrate Reductase Activity, RN: Root Nitrogen concentration, TNA: Total Nitrogen Accumulation.

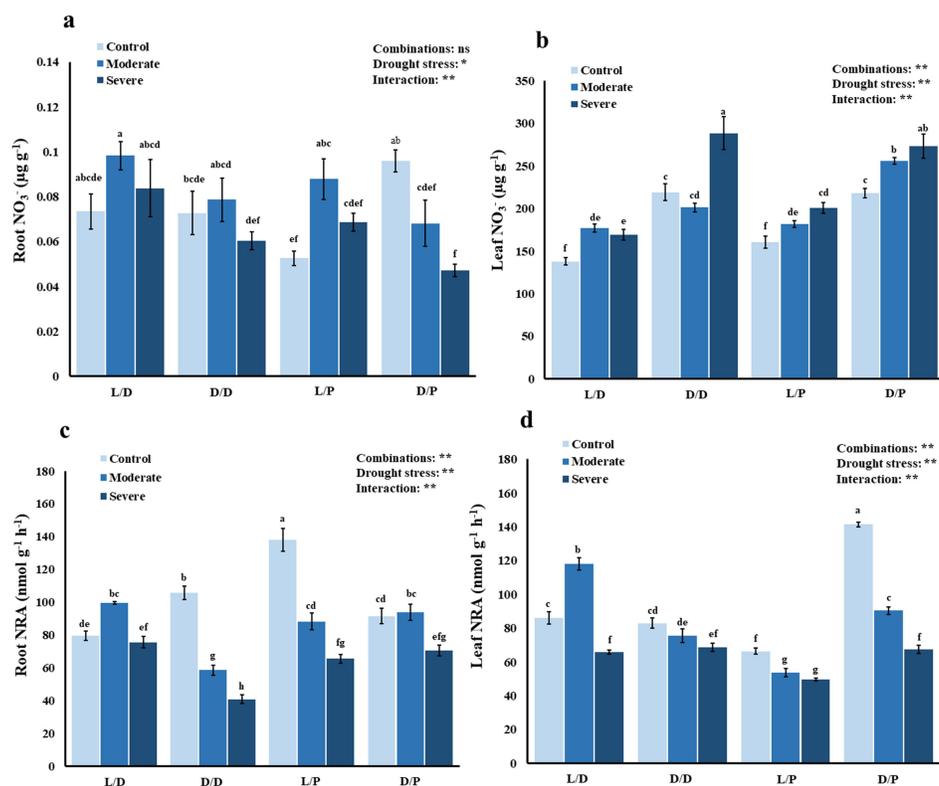


Figure 4. (a) Root NO₃⁻, (b) Leaf NO₃⁻, (c) Root nitrate reductase activity (NRA), and (d) Leaf nitrate reductase activity (NRA) of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different 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 ≤ 0.05 based on Duncan's multiple range test. ns, *, and **: Not significant, significant at P ≤ 0.05 and P ≤ 0.01, respectively.

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 5-c and -d).

DISCUSSION

In the present study, severe drought stress significantly reduced *TDW* and root-to-shoot ratio (Figures 3-a and -b). Our results indicate that 'Dargazi' 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 3-b). 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, among all four combinations, D/D and D/P had the highest Chl concentration, indicating that the 'Dargazi'

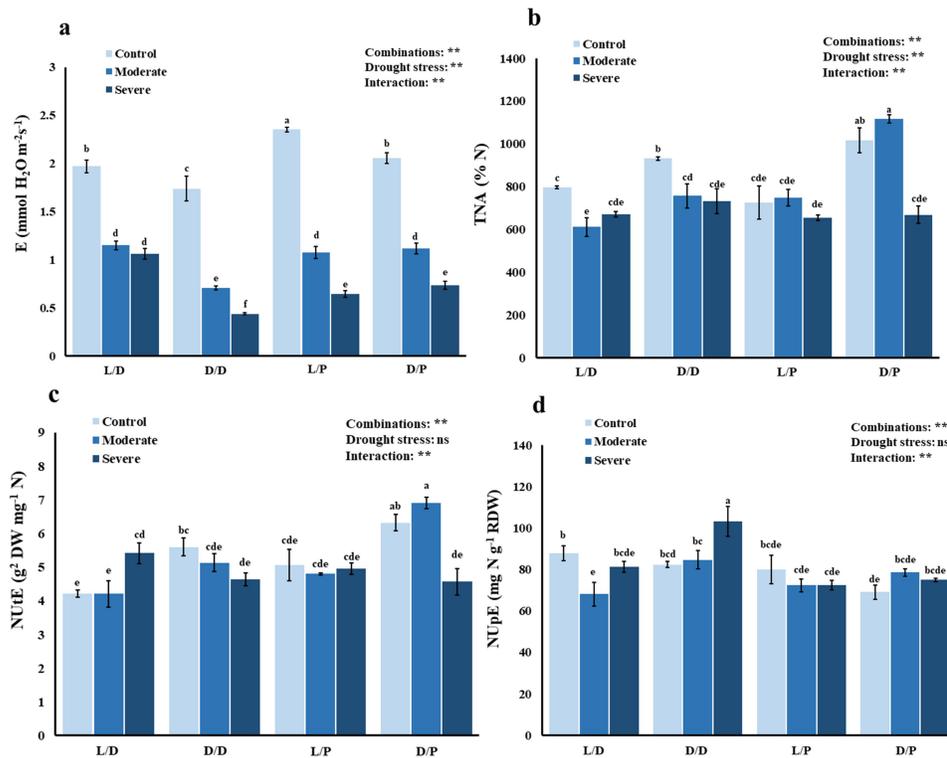


Figure 5. (a) Transpiration rate (E), (b) Total Nitrogen Accumulation (TNA), (c) Nitrogen Utilization Efficiency (NUtE), and (d) Nitrogen Uptake Efficiency (NUpE) of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') 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.

cultivar had more Chl concentration than 'Louise Bonne' (Figure 1-b).

Osmotic adjustment, water-use efficiency, chlorophyll concentration, leaf water potential, leaf relative water content, growth capacity, transpiration rate, and photosynthetic ability are the main indicators of plant growth and water status, especially under drought conditions (Qian *et al.*, 2020). An, PNUE, LRWC, SP, and EiC were not different between the four scion-rootstock combinations, but they showed a decrease under the influence of drought stress (Figures 2-a, -b, -c, -g, and -i). Rubisco and PEP carboxylase activity, known as the major limiting factors for photosynthesis under drought stress

conditions, as a result, causes a reduction in *EiC* and *An*, and there is a relationship between leaf *An* and *N* concentration as PNUE (Dinh *et al.*, 2017). Stomata are critical for regulating the uptake of CO₂ for photosynthesis and the evaporation of water for transpiration in plants, which makes them important regulators of WUE (Aliniaiefard *et al.*, 2020; Flexas *et al.*, 2004). K⁺ and Ca²⁺ have the main roles as significant inorganic osmoticum in stomatal opening and closure, because stomatal closure occurs via Ca²⁺ channels and inhibition of the flow of K⁺ ions into guard cells can be induced by ABA to control transpiration (Dayer *et al.*, 2020; Sussmilch *et al.*, 2017). We observed a positive

correlation of g_s and A_n with LCa^{2+} and a negative correlation with LK^+ (Table 5), which showed the role of K^+ and Ca^{2+} in stomatal conductance and photosynthesis rate by influencing stomata opening and closing. The SL/SW ratio and WUE_i were increased and the SA decreased by increasing stress levels (Tables 4 and 5). D/D and D/P had the lowest SA value and, subsequently, D/D showed the highest WUE_i . In addition, L/P showed the most increased SL/SW ratio compared to other scion-rootstock combinations in the present study. On the other hand, stomata act as ports that control the evaporation of water for transpiration and the uptake of CO_2 for photosynthesis. But, stomatal limitations are known as short-term responses to mild to moderate drought stress, whereas non-stomatal effects are usually considered to be more important during longer and more severe drought stress events (Roelfsema and Hedrich, 2002). However, A_n , C_i , and the E of the L/D and L/P leaves were significantly higher, indicating that the increase in the leaf photosynthetic efficiency in the 'Louise Bonne' cultivar may be a stomatal limiting factor (Wang *et al.*, 2018). Moreover, the aperture of stomata is a near-instantaneous, short-term response to environmental and endogenous signals, while the stomatal index and density are generally considered to be long-term developmental adaptations to the environment (Clavijo-Herrera *et al.*, 2018). There was no significant difference in stomatal density between the four combinations under drought stress conditions in our study (Table 3). We found that the levels of RK^+ and LCa^{2+} were increased with increasing stress levels (Figures 6-c and 2-f), and the LK^+ and RCa^{2+} were considerably decreased by increasing drought stress levels (Table 4 and Figure 6-d). The amount of LK^+ content was lowest in L/P and D/P combinations, indicating stomatal closure in scions on 'Pyrodwarf' rootstock was 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, A_n , 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 the control and moderate stress conditions (Figure 3-c). 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 variety-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 2-b and -g). The positive relationship between PNUE and A_n , g_s , E_iC , 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, and D/D had the lowest value of g_s and E (Tables 2 and 4, Figure 5-a). In addition, we found that C_i decreased by drought level increase (Table 4), and D/D and D/P showed lower values for C_i 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 1-a and 2-d). 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 1-a). 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 the control plants (Figure 6-b). That indicates 'Dargazi' as a cultivar and rootstock are efficient in 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 had the highest value of TNA (Figure 5-b). 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 had 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 observed 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 significantly decreased under severe stress conditions (Figures 4-a and -b). Apart from L/D that showed an increase under moderate stress, *NRA* in both leaf and root of all four scion-rootstock combinations considerably decreased by stress level increasing (Figures 4-c and -d). The high NO_3^- in leaves and its decrease 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 3-d). 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, which shows that 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 the main NUE parameters, we found *NUtE* and *NUpE*

were different between the 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 5-c 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).

CONCLUSIONS

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, 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 the 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.

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تأثیر تنش خشکی بر فیزیولوژی قسمت هوایی و جذب عناصر درشت مغذی در ترکیبات پایه - پیوندک گلابی (*Pyrus communis* L.)

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چکیده

تنش خشکی، یکی از تنش‌های غیر زیستی اصلی در مناطق خشک و نیمه خشک می‌باشد. مطالعه حاضر به بررسی چگونگی تأثیر تنش خشکی و پایه‌های مختلف بر فیزیولوژی اندام هوایی و درشت مغذی‌ها در دو نهال ارقام بذری درگزی و رویشی پیرودارف که ارقام درگزی و لوئیزبون بر روی آنها پیوند شده بودند می‌پردازد. تیمارهای آزمایشی شامل سه سطح آبیاری، آبیاری مناسب به عنوان شاهد (۱۰۰٪ ظرفیت مزرعه‌ای)، تنش آبی متوسط (۶۰ درصد ظرفیت مزرعه‌ای) و تنش آبی شدید (۳۰ درصد ظرفیت مزرعه‌ای) بود. طبق نتایج به دست آمده، تنش خشکی باعث کاهش نسبت وزن ریشه به اندام هوایی، محتوای آب نسبی برگ، مقدار پروتئین محلول، کارایی مصرف نیتروژن فتوسنتزی، پتانسیل اسمزی برگ، پتاسیم برگ، کلسیم ریشه، نیتروژن ریشه، دیافراگم روزنه، نرخ خالص فتوسنتز، سرعت تعرق، هدایت روزنه‌ای، و کارایی کربوکسیلاسیون آنی رویسکو در همه ترکیب‌های پیوندک- پایه گردید. همچنین، تحت تنش خشکی، سطوح پرولین، گلاسیسین بتائین، کربوهیدرات کل محلول، پتاسیم ریشه، کلسیم برگ، نیتروژن برگ، فسفر برگ و فسفر ریشه افزایش یافت. درگزی به عنوان رقم و پایه، راندمان مصرف آنی آب، راندمان استفاده از نیتروژن و راندمان جذب نیتروژن بالاتری داشت و همچنین مقادیر بالاتری از نیتروژن، نترات و

آمونیم برگ، پرولین، و پتانسیل اسمزی برگ را تحت تنش شدید خشکی نشان داد. رقم لوئیزبون مقادیر بالاتری از نسبت وزن ریشه به اندام هوایی، تجمع نیتروژن کل، فعالیت آنزیم نیترات ردوکتاز در برگ و ریشه، نرخ خالص فتوسنتز، مقدار دی اکسید کربن داخل سلولی، نرخ تعرق، و نیترات ریشه را داشت. و پایه پیروودارف تحت تنش خشکی متوسط بالاترین تجمع نیتروژن کل و کلسیم ریشه را متوسط نشان داد. این نتایج نشان داد که «درگزی» تحت استرس شدید تحمل بهتری از خود نشان داده، در حالی که پایه رویشی پیروودارف و رقم لوئیزبون در شرایط تنش متوسط پاسخ بهتری دارند.