

ACCEPTED ARTICLE

**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, 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

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

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

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47 1. Introduction

48 Fruit trees, unlike other plants, are generally composed of two different parts, the rootstock and
49 the scion growing together as one. In general, rootstocks as the tree's root system play an important
50 role, in influencing the whole plant's growth and development. Besides, nutrient uptake is adjusted
51 by its availability on the plant's root surface, which depends on the amount and movement of water
52 into the soil (Li *et al.*, 2013; Martinez *et al.*, 2020). The stomata affect the plant–water relations
53 and gas exchange, and drought stress can reduce nutrient uptake by the root, nutrient transport
54 from the root to the shoot, and partitioning in plants due to a restricted transpiration rate, limited
55 active transport, and reduced membrane permeability (Asayesh *et al.*, 2023b; Clavijo-Herrera *et*
56 *al.*, 2018; Sanchez-Rodriguez *et al.*, 2014). In addition, Drought stress influences the net carbon
57 assimilation rate, chlorophyll pigments, and photosynthesis, either by a direct effect of dehydration
58 on the photosynthetic apparatus or by an indirect effect by way of stomatal closure (Xia *et al.*,
59 2020). In plants, the transpiration rate determines the amount of calcium (Ca^{2+}) and potassium
60 (K^+) absorbed by the leaf (de Freitas and Mitcham, 2012). The osmotic adjustment (OA), as a tool
61 that lowers osmotic potential due to net solute accumulation in response to drought stress, by the
62 conservation of turgor during plant's water status changes may help to preserve the metabolic

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

69 European pear (*Pyrus communis* L.) is one of the most important commercial fruit tree species
70 that grow well in the temperate zones of the world and also in Iran (Arzani, 2021). The careful
71 selection and use of the seedling or clonal rootstock and the scion to rootstocks combinations are
72 critical for a better or proper orchard management system to lead to optimum crop yield, especially
73 under unfavorable environmental conditions (Arzani, 2017; Karbasi and Arzani, 2021). Therefore,
74 given the interactions that exist between the rootstock, the cultivar, and the environment, the
75 selection of scion-rootstock combinations that are affected by the rootstock root system is an
76 important task for a better orchard management system (Arzani, 1994; Arzani, 2017). Several
77 previous reports demonstrated the relationships between various important physiological
78 parameters of pear scion-rootstock combinations which can influence water relations, leaf gas
79 exchange, blooming, fruit set and quality, plant size, and yield efficiency (Asayesh *et al.*, 2023b;
80 Babaei *et al.*, 2021; Bosa *et al.*, 2014; Ikinç *et al.*, 2014). Trees enhancement vigor by the root
81 system of rootstock and its effect on plant yield could be increased water and nutrient uptake in
82 grafted plants. The mineral content value in aerial parts of the plant was attributed to the physical
83 characteristics of the root system such as lateral and vertical development in the rootstock, which
84 is one of the main reasons for the widespread use of grafted rootstocks (Sanchez-Rodriguez *et al.*,
85 2014; Seki *et al.*, 2007). Although cultivation of fruit crops is being shifted to the clonal rootstocks
86 in most fruit-growing countries, seedling rootstocks because of their deep root might be more
87 suitable rootstocks to be used under unfavorable growing conditions such as drought situations
88 (Arzani, 2017; Karbasi and Arzani, 2021). Besides, every rootstock responds differently to various
89 agro-climatic conditions, therefore, further study of clonal compared to seedling rootstocks is
90 necessary (Asayesh *et al.*, 2023a).

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

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

102

103 **2. Material and methods**

104 **2.1. Plant material**

105 We obtained two-year-old pear trees (*Pyrus communis* L.) from a commercial nursery in Karaj,
106 Iran. The trees were developed from 'Dargazi' (D) seedlings and in vitro propagated semi-dwarf
107 clonal 'Pyrodwarf' (P) rootstocks. The mature buds of commercially important scion cultivars,
108 'Dargazi' and 'Louise Bonne' (L), were grafted earlier onto the rootstocks, resulting in four different
109 rootstock-scion cultivar combinations (L/D, D/D, L/P, and D/P). All of the cultivars and rootstocks
110 are economically important locally. The developed trees were transferred into 12 L pots (one plant
111 per pot) during the dormant season, filled with loamy soil with the following characteristics: pH:
112 7.59, EC: 2.3 ds.m^{-1} , available nitrogen: 0.24%, available phosphorus: 110 mg kg^{-1} , and available
113 potassium: 717.6 mg kg^{-1} . The experiment was carried out under the research greenhouse
114 conditions, Department of Horticultural Science, Tarbiat Modares University, Tehran, Iran. The
115 research greenhouse received natural sunlight with a 10 to 14-hour photoperiod, 34/24 °C, and
116 45/60% average day/night temperature and relative humidity (%RH), respectively.

117

118 **2.2. Drought treatment application**

119 The experimental trees were allowed to grow for four months in the greenhouse and well-
120 watered conditions. Then, trees were subjected to the applied stress treatments for 60 days (August
121 to September 2020). The field capacity (FC) of the soil was determined by using the laboratory
122 pressure plate method (Ferrer *et al.*, 2008). The irrigation treatments were well-watered as control
123 (100% FC), moderate drought stress (60% FC), and severe drought stress (30% FC) with the soil
124 moisture content of 34.49%, 20.69%, and 10.34% after irrigation, respectively. To minimize the
125 surface evaporation effect, five pots without plants were used to determine evaporative water loss

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

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

132 2.3. *Morpho-physiologic characteristics*

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

136 The chlorophyll index was recorded using a SPAD chlorophyll meter (CCM-200, Opti-
137 Sciences, Inc., Hudson, NH, USA). Leaf relative water content (*LRWC*) was determined by the
138 method described by (Arzani, 1994; Wu *et al.*, 2008) as the following formula: $LRWC (\%) = (FW$
139 $- DW)/(TW - DW) \times 100$. Where *FW* is the fresh weight, *TW* is the turgid weight after rehydrating
140 samples for 24 h, and *DW* is the weight recorded after oven-drying samples at 85°C for 24 h.

141 2.4. Ψ_s and compatible solutes

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

145 The proline concentration of leaves was spectrophotometrically measured as described by
146 (Bates *et al.*, 1973). The absorbance of free proline content ($\mu\text{g g}^{-1}$ FW) was read at 520 nm, and
147 calculated based on a standard curve prepared with five standard concentrations (0–200 $\mu\text{g mL}^{-1}$)
148 of L-proline.

149 Glycine betaine (GB) content ($\mu\text{g g}^{-1}$ DW) was estimated according to the method of (Grieve
150 and Grattan, 1983). The absorbance was determined at 365 nm with a spectrophotometer
151 (SmartSpec Plus Spectrophotometer, BIO-RAD), and glycine betaine content was measured
152 according to a standard curve drawn from 0–200 $\mu\text{g mL}^{-1}$ glycine betaine as prepared in 2 M
153 sulfuric.

154 2.5. *TSC and SP*

157 Total soluble carbohydrates (TSC) were determined by the anthrone and reading absorbance at
158 625 nm based on the method described by (Vandoorne *et al.*, 2012). To quantify the total soluble
159 carbohydrates (mg g^{-1} FW) a calibration curve was established using glucose as the standard.

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

163

164 **2.6. Stomatal morphology**

165 To investigate the differences in stomatal morphology between pear scion-rootstock
166 combinations, the stomata of the lower epidermis on the new fully expanded leaf (abaxial surface)
167 from 4 randomly selected leaves and 80 stomata taken from each leaf were investigated by using
168 a light microscope (model Olympus BX51), according to the method described by (Asayesh *et al.*,
169 2021). Finally, ImageJ (U.S. National Institutes of Health, Bethesda, MD; <http://imagej.nih.gov/ij/>)
170 was used to measure the stomatal length/stomatal width ratio, stomatal aperture, and stomatal
171 density.

172

173 **2.7. Gas exchange measurements**

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

182

183 **2.8. K^+ , P and Ca^{2+} concentration**

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

189 AA). To measure phosphorus (P) content in leaves and roots, the colorimetric method of
190 molybdate-vanadate was used by spectrophotometers (model Optizen 3220UV). For this purpose,
191 2 ml of the extract prepared with ammonium molybdate-vanadate solution reached a volume of 15
192 ml and was used for reading at 470 nm to determine the amount of phosphorus.

193

194 **2.9. NO_3^- and NH_4^+ and NRA measurements**

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

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

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

219

220 **2.10. Total nitrogen concentration and NUE measurements**

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

- 226 - Total Nitrogen Accumulation (*TNA*) = N concentration × Total plant dry weight (mg N)
- 227 - Nitrogen Utilization Efficiency (*NUtE*) = Total plant dry weight / N concentration (g² TDW
228 mg⁻¹N)
- 229 - Nitrogen Uptake Efficiency (*NUpE*) = *TNA* / Root dry weight (mg N g⁻¹ RDW)

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

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

233

234 **2.11. Statistical analysis**

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

247

248 **3. Results**

249 **3.1. Morpho-physiologic characteristics**

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

253 considerably in L/D in moderate stress and D/D and D/P in severe stress compared to control
254 (Figure 3a). A decrease in the *R/SH* ratio was observed in D/D and D/P by increasing stress levels,
255 while L/D (1.3) and L/P (1.2) showed a higher *R/SH* ratio under moderate levels of drought stress
256 (Figure 3b). This indicates that all four scion-rootstock combinations had different responses to
257 drought stress at different levels. The Chlorophyll Index (*SPAD*), in the leaves of four pear scion-
258 rootstock combinations was significantly different. It was higher in D/D and D/P than in L/D and
259 L/P (Figure 1b). While *LRWC* markedly decreased under drought stress levels when compared to
260 that in the control. Reductions in moderate and severe drought stress conditions were 9.4 and
261 15.63% higher than in control conditions, respectively (Figure 2c).

262

263

264 **3.2. Ψ_s and compatible solutes**

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

277

278 **3.3. TSC and SP**

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

284

285 **3.4. Stomatal morphology**

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

295

296 **3.5. Gas exchange parameters**

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

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

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

322

323 **3.6. K^+ , P , Ca^{2+} concentration**

324 According to our results, the drought stress significantly influenced the macronutrient amount
325 in the leaf and root. The levels of LP , RK^+ (Figures 6b and c), RP , and LCa^{2+} (Figures 2e and f),
326 were increased with increasing stress levels. L/D and D/D had the highest amount of RK^+ and the
327 LP content in L/P under moderate and severe stress levels showed a huge increase when compared
328 to control plants. In contrast, the levels of LK^+ and RCa^{2+} were considerably decreased by
329 increasing drought stress levels (Table 2 and Figure 6d). The RCa^{2+} content of L/P and D/P
330 combinations was increased in moderate stress levels and LK^+ content was higher in L/D and D/D
331 combinations. The LK^+ presented a direct correlation with EiC , $PNUE$, and RN , as well as a high
332 negative correlation with LCa^{2+} , LNO_3^- , TSC , LN , and $Prol$ under drought stress conditions (Table
333 5).

334

335 **3.7. NO_3^- and NH_4^+ and NRA measurements**

336 The NO_3^- and NRA in leaf and root, were influenced by the scion-rootstock combinations and
337 drought stress levels interactions. The LNO_3^- was increased with increasing drought stress levels
338 and it was more in D/D and D/P in comparison to L/D and L/P (Figure 4b). In contrast, RNO_3^-
339 under moderate stress for L/D, D/D, and L/P changed with an increasing trend, and then all four
340 scion-rootstock combinations significantly decreased under severe stress conditions (Figure 4a).
341 In the case of NRA in both leaf and root, apart from L/D that showed an increase under moderate
342 stress, all four scion-rootstock combinations considerably decreased by stress level increasing. In
343 addition, NRA was different between the four scion-rootstock combinations, and in the control
344 condition, L/P (138) and D/P (141.3) had the highest NRA in root and leaf respectively (Figures
345 4c and d). The RNH_4^+ was significantly different between scion-rootstock combinations, and it
346 was higher in L/D and L/P than in D/D and D/P. and it was lower under severe drought levels in

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

350

351 **3.8. Nitrogen uses efficiency traits**

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

364

365 **4. Discussion**

366 In the present study, severe drought stress significantly reduced TDW and root-to-shoot ratio
367 (Figures 3a and b). Our results indicate that 'Dargazi' as cultivar had the highest amount of R/SH
368 ratio and as rootstock showed more reduction for R/SH ratio under severe stress. 'Louise Bonne'
369 cultivar on both rootstocks showed an increased R/SH ratio under moderate stresses (Figure 3b).
370 It has been reported that water stress causes differences in the production of roots and root dry
371 weight than shoot dry weight, which shows that plant species have different potential to develop
372 roots that have more excellent resistance to water stress (Jaleel *et al.*, 2009). In some situations,
373 drought caused a reduction in leaf morpho-physiological characterization like photosynthetic rate,
374 chlorophyll concentrations, dry biomass, and nitrogen assimilation, which resulted in low
375 carbohydrate production and poor nutrient metabolism and finally led to a reduction in growth
376 (Huang *et al.*, 2018b). Our results showed that between all four combinations, D/D and D/P had
377 the highest Chl concentration, indicating that the 'Dargazi' cultivar has more Chl concentration
378 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

410 considerably decreased by increasing drought stress levels (Table 4 and Figure 6d). The amount
411 of LK^+ content was lowest in L/P and D/P combinations, indicating stomatal closure in scions on
412 'Pyrodwarf' rootstock is more related to LK^+ induction than stomatal closure in scions on 'Dargazi'
413 rootstock. In contrast, compatible solutes like *Prol*, *GB*, and *TSC* were increased under drought
414 stress, and *Prol* was higher in D/D and D/P which had the highest stomatal closure (lowest g_s and
415 *SA*) (Table 1), and there was a negative relation between *Prol* and *SA*, *An*, g_s , and LK^+ (Table 5).
416 In addition, D/D and D/P showed the greatest decrease in osmotic potential under severe drought
417 stress compared to control and moderate stress conditions (Figure 3c). These results showed
418 stomatal movements in 'Dargazi' as a cultivar were more related to osmolytes than the 'Louise
419 Bonne' cultivar. Our results agreed with those of authors who indicated a higher *Prol*, *GB*, and
420 *TSC* accumulation under drought stress conditions, as a result, Ψ_s decreased which caused stomatal
421 closure (Asayesh *et al.*, 2017). According to previous studies, *GB* is a mediator of osmoregulation
422 and *Prol* is an important organic solute that plays a key role in the depot of energy to regulate
423 redox potential. Also, *TSC* can produce and accumulate osmolites to avoid the negative effects of
424 water stress, all known as the principal contributors to osmotic adjustment under osmotic stress
425 (Blum, 2017). Photosynthesis is a varieties-specific dependent factor that influenced growth
426 performance under stress. Besides, the concentration of soluble proteins may be used as an index
427 of Rubisco protein, because the content of this protein in leaves includes 30–50% of the total
428 soluble proteins (Sanchez-Rodriguez *et al.*, 2011), so drought stress also caused reductions in
429 *PNUE* and *SP* in our study (Figures 2b and g). The positive relationship between *PNUE* and *An*,
430 g_s , *EiC*, and *E*, indicates that with the decrease in the amount of gas exchange under drought stress
431 conditions, *PNUE* is also affected and decreased (Table 5). In our study, a decrease in g_s and *E* for
432 all scion-rootstock combinations was observed, D/D has the lowest value of g_s and *E* (Tables 2 and
433 4, Figure 5a). In addition, we find *Ci* decreased by drought level increasing (Table 4), and D/D
434 and D/P showed lower values for *Ci* under drought stress (Table 2). These results confirmed the
435 high capacity of 'Dargazi' as a cultivar or rootstock in conserving water loss under drought stress
436 by decreasing g_s and *E* and increasing *WUEi*. Transpiration is the main reason for water loss in
437 plants. Generally, under drought stress conditions, stomatal conductance and transpiration rate are
438 significantly reduced, to maintain the leaf's relative water content (*LRWC*). In line with the current
439 results, previous studies presented a significant reduction in growth traits and many physiological

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

442 Earlier research revealed that drought conditions generally decrease the LK^+ content, without
443 effect on the *N* and *P* contents in apple trees (Ferree and Schmid, 2000); however, drought stress
444 decreases the *P*, *N*, and K^+ contents in leaves of *Malus hupehensis* (Liang *et al.*, 2017). In the
445 current study, we found a decrease in *N* concentration by increasing drought stress in the root, but
446 not in the shoot (Figures 1a and 2d). The *LN* concentration was different between scion-rootstock
447 combinations, and it was more in D/D and D/P than in L/D and L/P (Figure 1a). The levels of *LP*
448 and *RP* were increased with increasing stress levels and the *LP* content in L/P under moderate and
449 severe stress levels showed a huge increase when compared to control plants (Figure 6b). That
450 indicates 'Dargazi' as a cultivar and rootstock are efficient to *N* uptake more than 'Louise Bonne'.
451 The positive correlation between *WUEi* and *LN* (Table 5) shows that under drought stress 'Dargazi'
452 has high water and nitrogen accumulation in leaves. On the other hand, *TNA* had the highest
453 decrease in D/D and D/P, especially under severe stress levels; also, L/P and D/P under moderate
454 stress levels have the highest value of *TNA* (Figure 5b). These results indicate that the 'Dargazi'
455 cultivar has a higher *N* accumulation in the leaf than in 'Louise Bonne'. Nevertheless, *TNA* increase
456 under moderate stress showed that 'Pyrodwarf' rootstock has more ability to absorb and transfer *N*.
457 *TNA* decreasing shows that the limitation of g_s and *E* under drought stress limits *N* uptake because
458 *N* is necessary for regulating the adaptation of photosynthesis to water stress. Drought can affect
459 nitrogen absorption and transport to the aboveground plant portions by decreasing the transpiration
460 rate and membrane permeability (Iqbal *et al.*, 2020). We saw high NO_3^- concentration for D/D
461 and D/P in comparison to L/D and L/P in the leaf. The roots NO_3^- under moderate stress for L/D,
462 D/D, and L/P changed with an increasing trend, and then significantly decreased under severe
463 stress conditions (Figures 4a and b). *NRA* in both leaf and root, apart from L/D that showed an
464 increase under moderate stress, all four scion-rootstock combinations considerably decreased by
465 stress level increasing (Figure 4c and d). The high NO_3^- in leaves and decrease of it in roots under
466 severe stress levels may be due to high or low *NRA* in plants because there is a negative correlation
467 between *NRA* and NO_3^- concentration and the activity state of *NR* is very dependent on water
468 status (Abenavoli *et al.*, 2016). Under drought stress, the leaf, and root NH_4^+ showed a decreasing
469 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
470 highest value in D/P (Figure 3d). All of these results confirm that 'Dargazi' has high LNO_3^- and

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

492 493 5. Conclusion

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

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

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

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665 **Table 1.** Stomatal aperture (*SA*), stomatal length/width ratio (*SL/SW* ratio), stomatal conductance
 666 (*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</i> × <i>b</i> | 0.078 ^{ns} | 0.023 ^{ns} | 0.006 ^{ns} | 7.06 ^{ns} |

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

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672 **Table 2.** Intracellular CO_2 concentration (*C_i*), instantaneous water use efficiency (*WUE_i*), leaf K^+ ,
 673 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\times<i>b</i></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.
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687 **Table 4.** Effect of three levels of drought stress (Control, Moderate, and Severe) on C_i , intracellular
688 CO_2 concentration (*C_i*), instantaneous water use efficiency (*WUE_i*), leaf K^+ (*LK⁺*), and root NH_4^+
689 (*RNH₄⁺*) of four different pear scion-rootstock combinations.

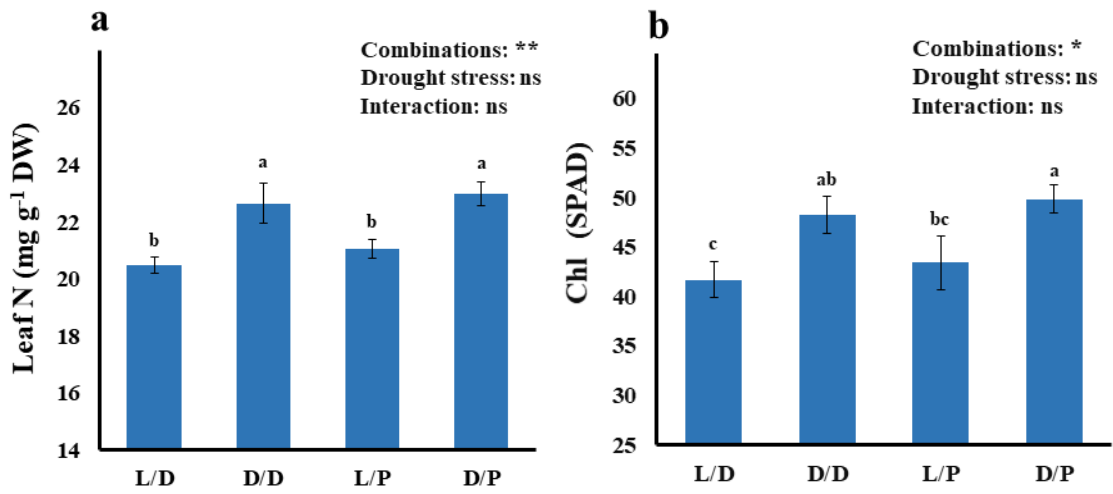
| Drought level | <i>C_i</i> ($\mu\text{mol CO}_2 \text{ mol}^{-1}$) | <i>WUE_i</i> ($\mu\text{mol CO}_2 / \text{mmol H}_2\text{O}$) | <i>LK⁺</i> (%) | <i>RNH₄⁺</i> ($\text{mg g}^{-1}\text{DW}$) |
|-------------------------------------|---|--|------------------------------|---|
| Control | 237.8 \pm 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\times<i>b</i></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.
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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>LCa²⁺</i> | <i>LNO₃⁻</i> | <i>LNH₄⁺</i> | <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>LCa²⁺</i> | ** | ** | | ** | | ** | ** | ** | * | ** | ** | * | * | 1 | | | | | | | |
| <i>LNO₃⁻</i> | | | ** | | ** | | | | ** | | | | * | | 1 | | | | | | |
| <i>LNH₄⁺</i> | * | | | | | | | | | | | | | | | 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, *EiC*: 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 *LCa²⁺*: leaf calcium, *LNO₃⁻*: leaf nitrate, *LNH₄⁺*: leaf ammonium, *LNRA*: leaf nitrate reductase activity, *RN*: root nitrogen concentration, *LN*: leaf nitrogen
701 concentration, *TNA*: total nitrogen accumulation.
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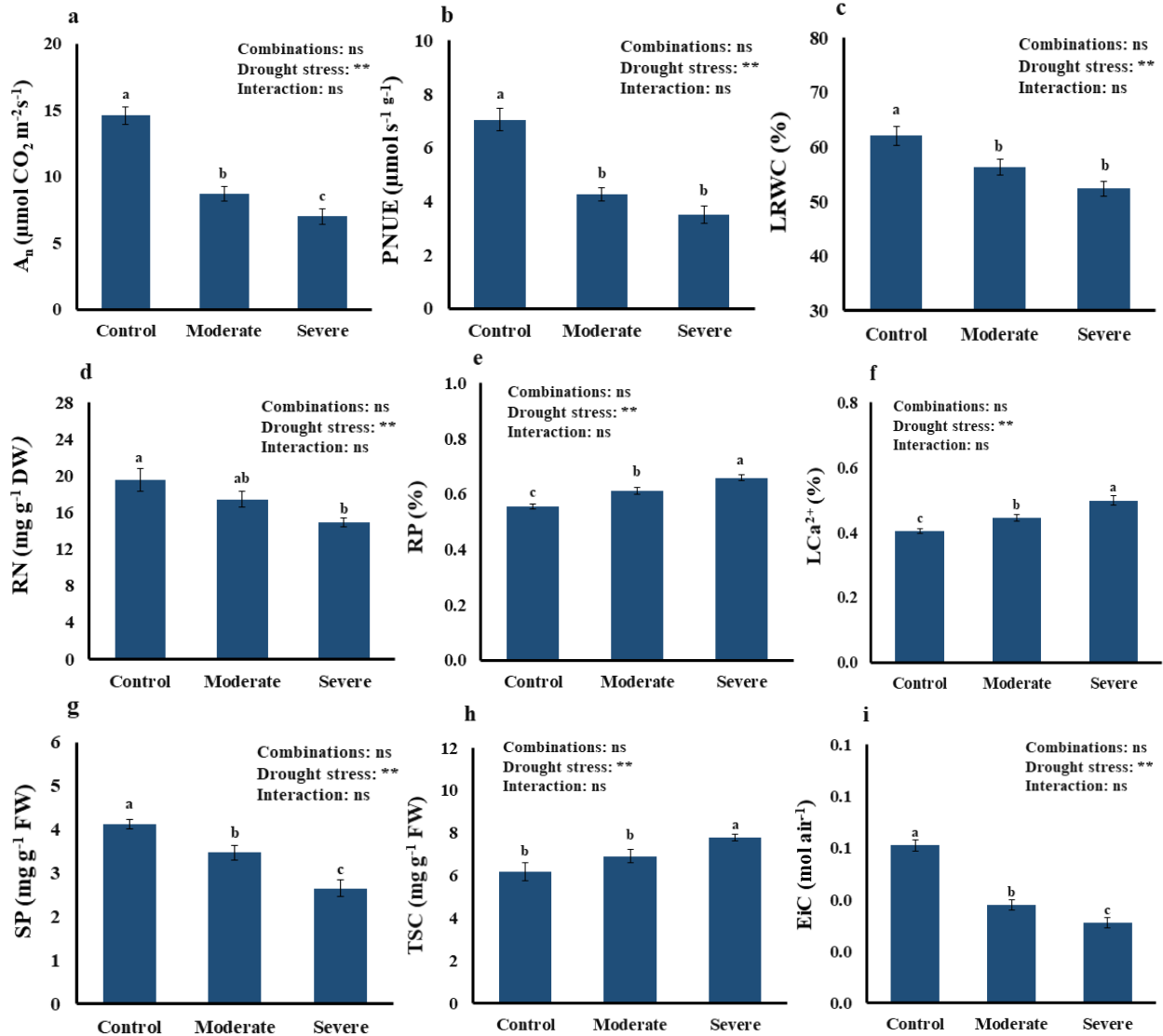
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Figure 1. Leaf nitrogen concentration (a), and chlorophyll concentration (b) of four different pear scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf'). 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.



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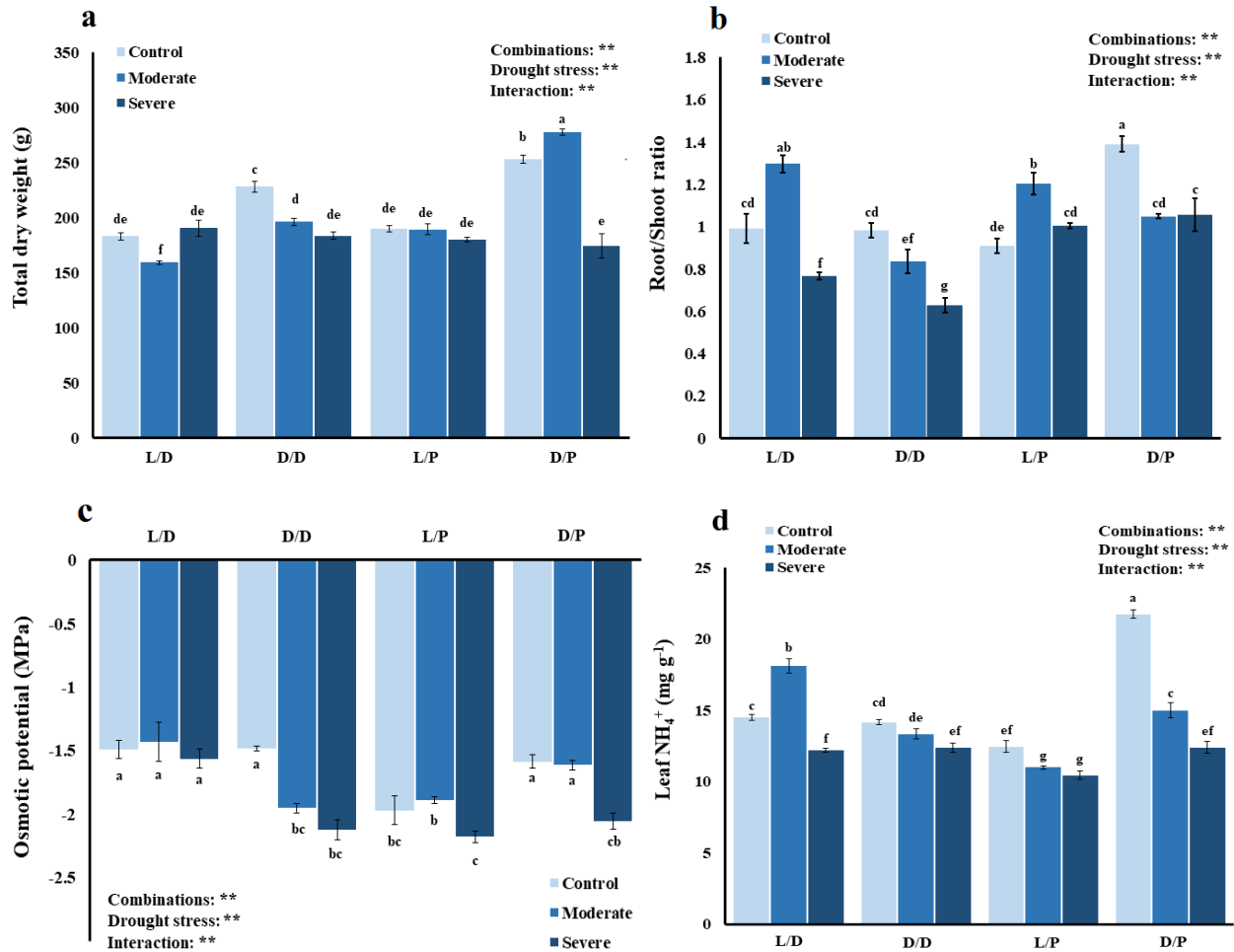
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Figure 2. Effect of three levels of drought stress (Control, Moderate, and Severe) on net photosynthetic rate (a), photosynthetic nitrogen use efficiency (b), leaf relative water content (c), root nitrogen concentration (d), root phosphorus content (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.

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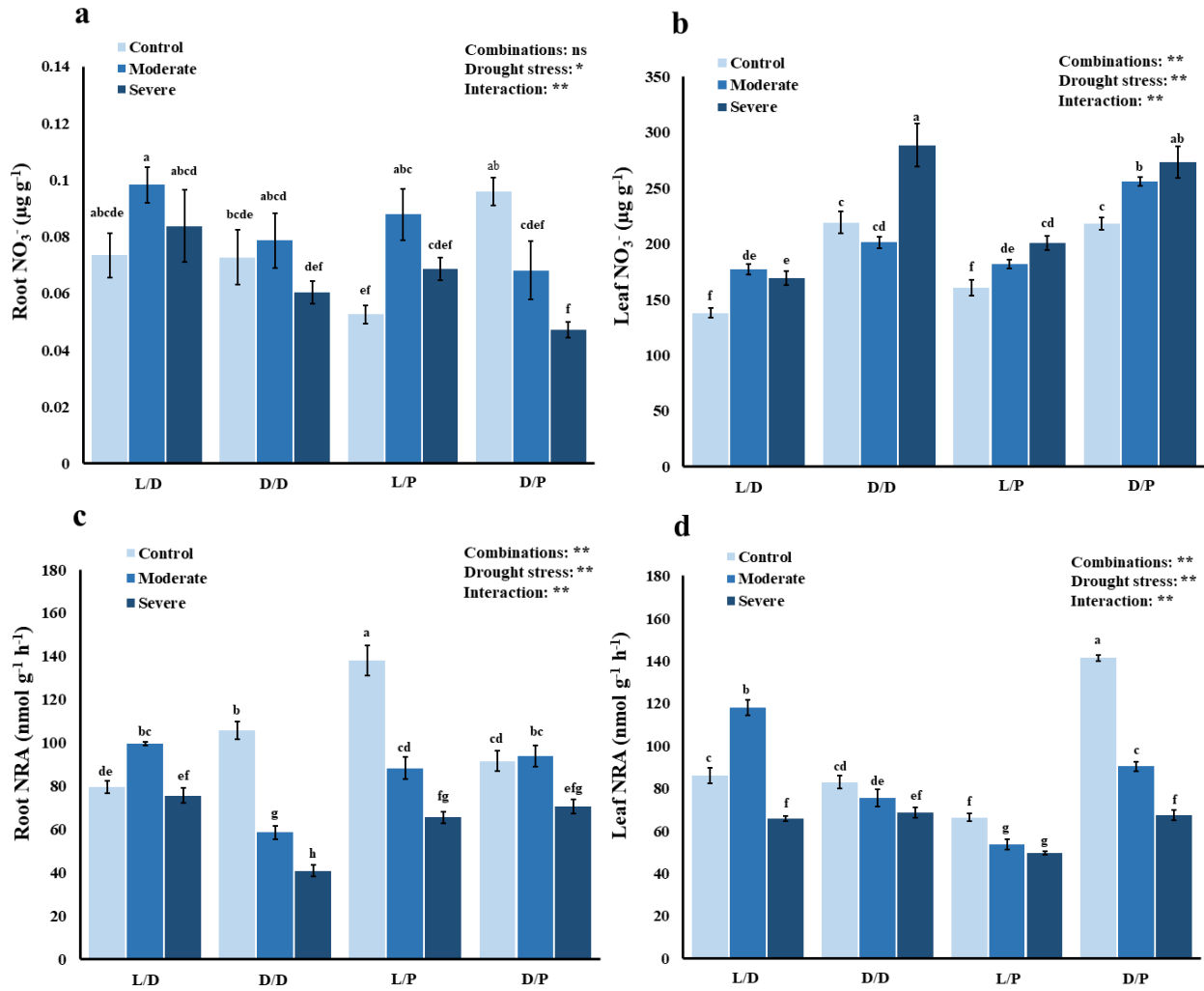
728 **Figure 3.** a: total dry weight (*TDW*), b: root-to-shoot ratio, c: osmotic potential (Ψ_s), d: leaf NH_4^+
 729 of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi',
 730 L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf') under three different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

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741 **Figure 4.** a: root NO_3^- , b: leaf NO_3^- , c: root nitrate reductase activity (NRA), d: leaf nitrate

742 reductase activity (NRA) of four scion-rootstock combinations (L/D: 'Louise Bonne'-'Dargazi',

743 D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P: 'Dargazi'-'Pyrodwarf')

744 under three different drought stress conditions (Control, Moderate, Severe). Vertical bars represent the \pm

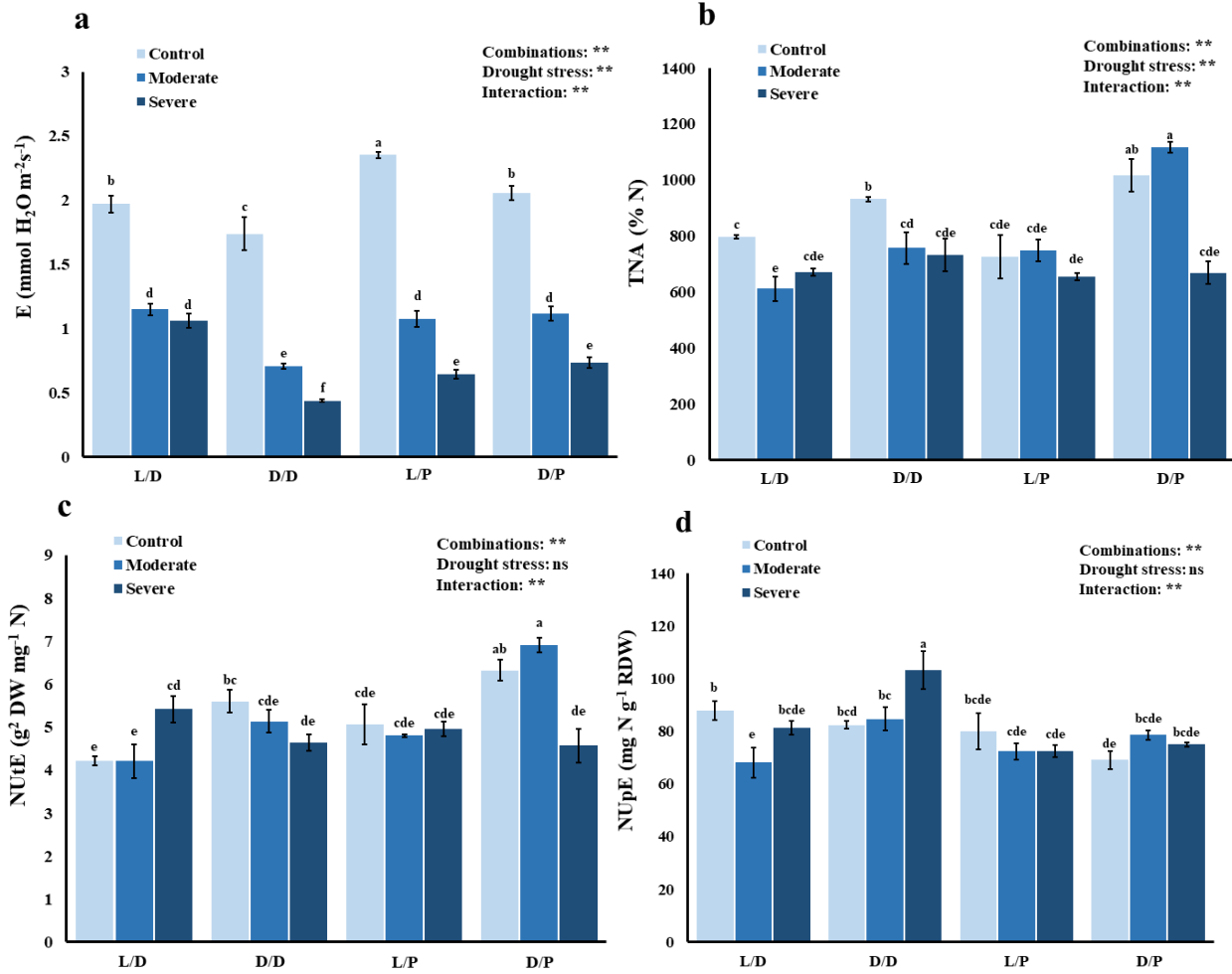
745 standard error of the mean (n=4). Different letters above individual bars indicate statistically

746 significant differences among treatments at $P \leq 0.05$ based on Duncan's multiple range test. ns, *,747 and **: not significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

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751 **Figure 5.** a: transpiration rate (E), b: total nitrogen accumulation (TNA), c: nitrogen utilization752 efficiency ($NUtE$), d: nitrogen uptake efficiency ($NUpE$) of four scion-rootstock combinations

753 (L/D: 'Louise Bonne'-'Dargazi', D/D: 'Dargazi'-'Dargazi', L/P: 'Louise Bonne'-'Pyrodwarf', D/P:

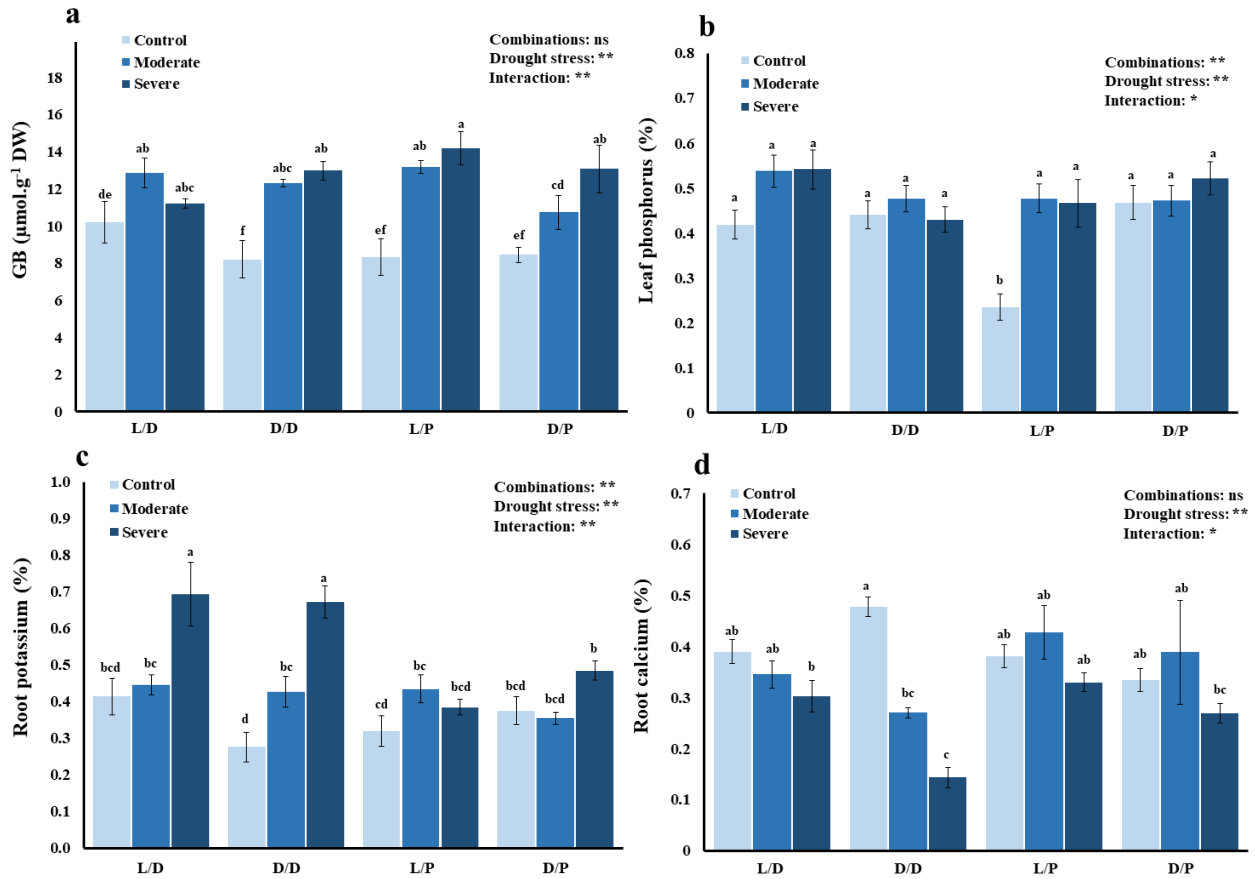
754 'Dargazi'-'Pyrodwarf') under three different drought stress conditions (Control, Moderate, Severe).

755 Vertical bars represent the \pm standard error of the mean ($n=4$). Different letters above individual756 bars indicate statistically significant differences among treatments at $P \leq 0.05$ based on Duncan's757 multiple range test. ns, and **: not significant, and significant at $P \leq 0.01$, respectively.

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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.