Acta Sci. Pol. Hortorum Cultus, 19(2) 2020, 105–121

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2020.2.11

ORIGINAL PAPER

Accepted: 4.07.2019

VARIATIONS IN GROWTH AND PHOTOSYNTHETIC PARAMETERS OF SOME CLONAL SEMI-DWARFING AND VIGOROUS SEEDLING PEAR (*Pyrus* spp.) ROOTSTOCKS IN RESPONSE TO DEFICIT IRRIGATION

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ABSTRACT

A greenhouse experiment was conducted to estimate the impacts of various deficit irrigation regimes (DIR) as 100 (control), 75 (medium DIR, MDIR) and 50% (severe DIR, SDIR) of field capacity (FC) on the growth and photosynthetic traits of five rootstocks (two vigorous seedling including *Pyrus betulifolia* and Dargazi seedling (*P. communis* L.) as well as three clonal semi-dwarfing rootstocks including OH×F69, OH×F87 and Pyrodwarf in 2016. Although in all studied rootstocks the growth and photosynthetic parameters negatively affected under MDIR and SDIR conditions, but *P. betulifolia* and Pyrodwarf rootstocks had the better growth rate, chlorophylls, total carbohydrates, relative water content, sub-stomatal CO_2 (C_i), photosynthesis rate (P_N) and instantaneous water use efficiency compared to others. Therefore, the use of *P. betulifolia* and Pyrodwarf (as drought tolerance rootstocks) could be more appropriate under various DIR conditions.

Key words: carbohydrates, chlorophylls drought tolerance, *P. betulifolia*, Pyrodwarf

Abbreviations: DIR – deficit irrigation regimes, MDIR – medium deficit irrigation regimes, SDIR – severe deficit irrigation regimes, FC – field capacity, LRWC – leaf relative water content, C_i – leaf internal (sub-stomatal) CO_2 concentration, E – transpiration rate, g_s – stomatal conductance, P_N – net photosynthesis rate, WUE – instantaneous water use efficiency, WUEi – intrinsic water use efficiency, FW – fresh weight, TW – turgid weight, DW – dry weight, TCC – total carbohydrates content

INTRODUCTION

One of the main detrimental factors that affect almost 45 percentages of the world's agricultural regions is drought stress. Drought restrict the availability of water to absorb by plant root cells; which significantly disturb plant growth traits and also many physiological functions like stomata regulation, photosynthesis and carbohydrates accumulation [Ahmad 2016, Vats 2018].

Photosynthesis is a fundamental process, which needs an ideal equilibrium between carbon fixation, sucrose production and sucrose distribution between plant tissues [Mishra 2004]. It is obviously impaired by drought stress via changed in plant water potential, stomata closing, the declined CO_2 diffusion to the location of carboxylation, photo-damage to photosystem II (PSII), reduction of the leaf area and the

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chlorophyll content, metabolic restrictions and also prevention of plant growth [Siddique et al. 2016]. This prevention diminishes the recycling of inorganic phosphate between the cytosol and the chloroplast, resulting in slight photosynthetic rates. The decline in net photosynthesis may cause a reduced transport of crucial metabolites to the sources. During water scarcity situations the content of leaf changed straightly by water shortages or indirectly through declined growth [Pinheiro and Chaves 2011, Siddique et al. 2016]. On the other side, dryness conditions may aggravate oxidative stress/ oxidative signaling in plants. Oxidative stress is recognized to directly and/or indirectly impact the biosynthetic pathways of several compounds. These mechanisms of drought stress seem strictly associated because the accumulation and aggregation of compounds like carbohydrates may exacerbate photo-oxidative damage in photosynthetic tissues [Ripoll et al. 2014].

Considering this case, to prevail with the difficulties regarding water shortage farmers should change their irrigation techniques by applying approaches to water saving. Among the approaches that might be utilized to achieve water saving are the application of improved, advanced and accurate DIR capable to diminish the influence on plant growth attributes. Furthermore, to assistance to water reserving, tree culture must be focused on the usage of species/cultivars that are lower water-requesting or able to endure DIR with the least influence on the fruit quality parameters [Galindo et al. 2018, García-Tejero et al. 2018].

Pear (*Pyrus spp.* L.), which is part of the *Rosaceae* family, subfamily *Spiraeoideae* is the third most important commercial fruit trees in temperate zones [Wu et al. 2018]. Pear is an appropriate source of sugar, vitamins, carbohydrates, fibers and also other nutritional compounds that keep healthier weights [Li et al. 2016]. In the commercial pear orchards, to achieve trees that are more endurance to several biotic and abiotic stresses, various pear rootstocks previously utilized. The Pyrus genus is genetically diverse with one thousand variety that makes a good source to select the appropriate rootstock under normal and stressful conditions [Campbell 2003, Sharma and Sharma 2008].

Among pear rootstocks, *P. communis* L., *P. calleryana* Decne and *P. betulifolia* are commonly applied rootstocks that grow vigorously and therefore producing large pear trees that in several cases have highly productive and are compatible with most pear varieties [Einhorn et al. 2013, Ikinci et al. 2014]. On the other side, the progress and adoption of dwarfing and/or semi-dwarfing rootstocks have developed the efficiency of tree fruit cultivation due to rise in planting density, decline the juvenility term and costs, enhance of biotic and abiotic stress endurance, increase precocity and also higher fruit quality [Ou et al. 2015].

Quince A (EMA) and Quince C (EMC) rootstocks, which belong to *Cydonia oblonga* Mill, and better tree uniformity and lower vegetative growth characteristics, have become abundantly commercialized in many pear producing areas. Moreover, vigorous seedling rootstocks and also some clonal semi-dwarfing rootstocks belong to *P. communis* L. such as Pyrodwarf, OH×F69 and other OH×F rootstocks have been recently used in some orchards commercially in West Asian countries with more limiting soil conditions [Campbell 2003, Necas and Kosina 2008].

Recently, some studies reported the effects of some rootstocks on photosynthetic productivity [Losciale et al. 2008, Bosa et al. 2016], growth, fruit attributes and nutrient element levels [Ikinci et al. 2014], agronomical efficiency and fruit quality [Iglesias and Asin 2011, Iglesias and Batlle 2011] in pears cultivars. Furthermore, the influence of DIR on the growth and water potential [Sun et al. 2016], vegetative and generative parameters [Cheng et al. 2012], yield and fruit quality [Janssens et al. 2011], yield and tree water status [Marsal et al. 2010] of pear tree previously have been revealed.

However, there is rare or no reliable information about the influence of different rootstocks under various DIR regimes on the photosynthetic characteristics of pear. Therefore, the current study was performed for primary screening and comparison of the five rootstocks including seedlings of *P. betulifolia* and *P. communis* (from cultivar 'Dargazi') and three semi-dwarfing clonal rootstocks [Pyrodwarf (Rhenus 1), OH×F69 (Daynir) and OH×F87 (Daytor)] on the growth and photosynthesis parameters of pear under various DIR.

MATERIALS AND METHODS

Rootstock materials. In this research, the impacts of two vigorous pear rootstocks including seedlings of *P. betulifolia* and *P. communis* (from cultivar 'Dargazi') and three semi-dwarfing clonal rootstocks, Pyrodwarf (Rhenus 1), OH×F69 (Daynir) and OH×F87 (Daytor) were evaluated under different DIR regimes in 2016. The seeds of *P. betulifolia* were achieved from Florsilva Ansaloni (San Lazzaro Di Savena, Italy) and Dargazi seedlings were obtained from Pear National Collection of the Temperate Fruit Research Center of the Horticultural Sciences Research Institute of Iran. To homogeneous propagation of the seedling rootstocks, following seeds germination (after 8 weeks stratification at 4°C), one seedling were micropropagated on the modified QL [Leblay et al. 1991], enriched by 3% w/v sucrose, 4.4 mM 6-benzylaminopurine, 0.5 mM naphthalene acetic acid and 0.6% w/v plant agar (Duchefa, The Netherlands). The pH of all media was adjusted at 5.7 ±0.1 before addition of agar and autoclavation. The plant materials were grown *in vitro* under 16 h light photoperiod, using cool white fluorescent lamps at 40 μmol m^{-2} s^{-1} photon flux by using cool white fluorescent lamps (Sylvania, Germany) at a stable temperature of 23 ±1°C. It should be noted that the shootlets were subcultured every 6 weeks.

Similarly, all three clonal Pyrodwarf, OH×F69 and OH×F87 rootstocks were prepared as virus-free plant materials and micropropagated on the same media and growth conditions as described for seedling rootstocks. Proliferated shootlets of both vigorous and semi-dwarfing rootstocks were used as micro-cuttings, and established for 3–4 weeks on the modified QL, enriched by 3% w/v sucrose, 1 mg L^{-1} indole-3-butyric acid and 0.6% w/v plant agar (Duchefa, the Netherlands) for root induction [Leblay et al. 1991, Abdollahi et al. 2004]. The micro-cuttings were transferred to the plant growth regulator-free media to root elongation for 4 weeks. At least 50 plantlets of each rootstock were acclimatized in 50% coco peat/ 50% perlite (v/v) soil mixture in greenhouse condition with at least 80–90% humidity for 12 weeks.

Finally, 10 well-grown plans with at least 40–45 cm height, 10–15 cm long roots, and expanded leaves from each rootstock were potted in 10 L containers and then moved to secondary greenhouse with 27–35°C day, 20–25°C night temperatures and 30% relative humidity (as a normal humidity in the summer). The containers mixture was prepared homogenously by the soil of Horticultural Research Station of Temperate Fruit Research Center, with the soil physicochemical conditions including clay loam texture, pH = 7.8 and 0.8% total organic carbon.

Deficit irrigation (DI) treatments. DI treatments were performed according to the relative maintaining of the soil moisture based on the FC of the containers mixture. Three levels of DIR including 100 (as control), 75 (as medium DIR, MDIR) and 50% (as severe DIR, SDIR) of the FC were continued for all nine replications of vigorous and semi-dwarfing pear rootstock during 60 days from 6 July to 7 September 2016. Throughout the experimental period, the lost water of the treatments was measured by weighing the pots diurnal and replacing the lost water content. The drought-stress treatments started after 10 days establishment of rootstock in the greenhouse and when all rootstocks had at least 50 leaves and 40–45 cm in height.

Characteristics measurement. The growth characteristics of rootstocks were measured every 15 days during growing season. Leaf relative water content (LRWC) and total carbohydrates content (TCC) of pear leaves were assayed at two stages (30 and 60 days after DIR). LRWC was determined according to Turner [1986], as fully expanded leaves were collected from each rootstock at each analysis time and weighed immediately as fresh weight (FW). Leave samples kept 4 h in distilled water and weighed again as turgid weight (TW). Then samples oven-dried at 70°C for 24 h and weighed again as dry weight (DW). Finally, LRWC was calculated using following equation [Rigosa and González 2001]:

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

Where FW is sample fresh weight, TW is sample turgid weight and DW is sample dry weight. Percentage of leaf dry matters was measured at the end of experiment with nine replications.

TCC of the leaves were analyzed according to the colourimetric method with anthrone reagent [Leyva et al. 2008]. As a central photosynthetic pigment, chlorophylls content (chlorophyll *a*, chlorophyll *b* and total chlorophyll) of the leaves were assayed as presented by Moran [1982], after extraction with N,N-dimethylformamide at 4°C and kept in a refrigerator overnight in the darkness. Photosynthetic parameters including sub-stomatal CO_2 (C_i), stomatal conductance (g_s), photosynthesis rate (P_N) and transpiration rate (E), were analyzed by using LC*i*-SD (ADC, Bioscientific Ltd., UK) based on the Gago et al. [2016] method. Chamber and leaf temperatures were evaluated by the same instrument on 10 leaves in each rootstock. Instantaneous water use efficiency (WUE) was calculated as P_N/E [Podlaski et al. 2017], and the intrinsic water use efficiency (WUEi) was determined as P_N/g_s [Yin et al. 2006].

Data Analysis. The pots of rootstocks were arranged in a factorial experiment according to a randomized complete block design (RCBD) with nine individual replications. Variation of the growth, biochemical and photosynthetic factors were compared by drawing of curves, using Microsoft Excel (Microsoft, USA-Version 2007) and mean comparison following ANOVA and Duncan's Multiple Range Test (DMRT) by Microsoft SigmaPlot (Sigma-Aldrich, USA-Version 11.5). Rootstocks growth rate (RGR) under various DIR were compared by calculating $tg\alpha_{gr}$, determined as Δ height/between two evaluated times (0 and 60 days). Similarly, the rate of chlorophyll loss in the leaves under various DIR was compared by calculating $tg\alpha_{chl}$, determined as Δ chlorophyll loss/between 100–50% of FC.

RESULTS

Rootstocks growth rate (RGR). The results revealed that RGR significantly influenced by various DIR and also different rootstock (Fig. 1). In the normal situations (100% of FC), the two vigorous rootstocks (*P. betulifolia* and Dargazi seedling) had the highest growth rates with 0.69 and 0.58 of $tg\alpha_{gr}$ among semi-dwarfing rootstocks. Under MDIR (75% of FC) and SDIR (50% of FC) conditions, the RGR significantly declined, as the OH×F87, Dargazi seedling, OH×F69, Pyrodwarf and *P. betulifolia* respectively showed the highest RGR reduction (Fig. 1). Moreover, only in *P. betulifolia* rootstock, significant differences were observed in the RGR between 75 and 50% of DIR, which suggest this rootstock had more gradable tolerance strategy against water restriction situation.

Total carbohydrates content (TCC). As shown in Table 1, in all rootstocks, TCC of leaves showed a regular enhancing under various DIR and also after 60 days as compared with first evaluated time. This increment was more than 1% in *P. betulifolia* and less than 0.3% in OH×F87, that have shown the highest and the lowest RGR under various DIR. Moreover, after 60 days and under SDIR situation, no significant difference was obtained between rootstocks expect in Dargazi seedling, which had the lowest TCC of leaves compared to others (Fig. 2).

Leaf relative water content (LRWC). It was indicated that LRWC significantly reduced in response to increasing of DI severity, as the highest LRWC was obtained in control plants, whereas plants grown in SDIR situation had the lowest LRWC (Tab. 1). At the first evaluated time, there was no significant difference between all rootstocks under each DIR (Fig. 2), but after 60 days, Pyrodwarf showed the highest leaf LRWC in control situation and also under MDIR and SDIR conditions.

Leaf temperature. The findings of our experiment mentioned that rootstocks and various DIR significantly influenced the leaf temperature (Tab. 1). Change in leaf temperature between two evaluated times depended on rootstocks, as leaf temperature decreased in *Pyrus betulifolia,* Pyrodwarf, while Dargazi seedling, OH×F69 (Daynir) and OH×F87 (Daytor) had no significant changes compared to the first evaluated time. Moreover, in both evaluated times, leaf temperature significantly enhanced along with increasing of DI severity (Tab. 1). As shown in Figure 2, in both evaluated times *Pyrus betulifolia* had the lowest leaf temperature in various DIR as compared with other rootstocks.

Chlorophyll contents. Under normal situation (100% of FC), total chlorophyll content of rootstocks belong to *P. communis* species including Dargazi seedling, OH×F69 and OH×F87 were about 3.0 mg g^{-1} FW, while in *P. betulifolia* and Pyrodwarf total chlorophyll content was considerably less and about 2.6 mg g^{-1} FW (Fig. 3). In all rootstocks, chlorophylls content (chlorophyll *a*, chlorophyll *b* and total chlorophyll) significantly diminished under MDIR and SDIR conditions. According to the $tg\alpha_{chl}$, this declining was higher in OH×F87 (Daytor), Dargazi Seedling and OH×F69 (Daynir), respectively (Fig. 3). Furthermore, *P. betulifolia* and Pyrodwarf had the lowest declining rates of chlorophylls content with $tg\alpha_{chl}$ = 0.0157 and $tg\alpha_{chl}$ = 0.0171. These results mentioned that *P. betulifolia* and Pyrodwarf were able to maintain the highest chlorophyll *a*, *b* and total chlorophyll content under various water shortage situations.

Photosynthetic parameters. As shown in Table 2, all evaluated photosynthetic parameters (C_i, E, g_s, P_N, P_N/E and P_N/g_s) significantly influenced by rootstocks and also various DIR. It was revealed that C_i, E, g_s, P_N and P_N/E significantly declined along with increasing of DI severity, as rootstocks under SDIR conditions showed the lowest C_i, E, g_s, P_N and P_N/E amount compared to normal situation, whereas P_N/g_s amount significantly enhanced from 38.47 µmol mol^{-1} to 51.49 and 55.94 µmol mol^{-1} under MDIR and SDIR conditions, respectively.

The interaction impacts of rootstocks and DIR mentioned that under MDIR and SDIR, the highest C_i amount was obtained in *P. betulifolia* and Pyrodwarf (Fig. 4). After 60 days, under various DIR the lowest E content was found in *P. betulifolia* but had no significant difference with other rootstocks. Although, *P. betulifolia* and Pyrodwarf mentioned the highest g_s amount under various DIR, but had no significant difference with other rootstocks. As shown in Figure 4, under MDIR and SDIR conditions *P. betulifolia* and Pyrodwarf had the highest P_N amount as compared others. On the other side, the highest instantaneous WUE observed in *P. betulifolia*, Pyrodwarf and OH×F69 (Daynir), respectively. Finally, after 60 days, the lowest WUEi was found in *P. betulifolia*, Pyrodwarf and OH×F69 (Daynir) under SDIR conditions (Fig. 4).

DISCUSSION

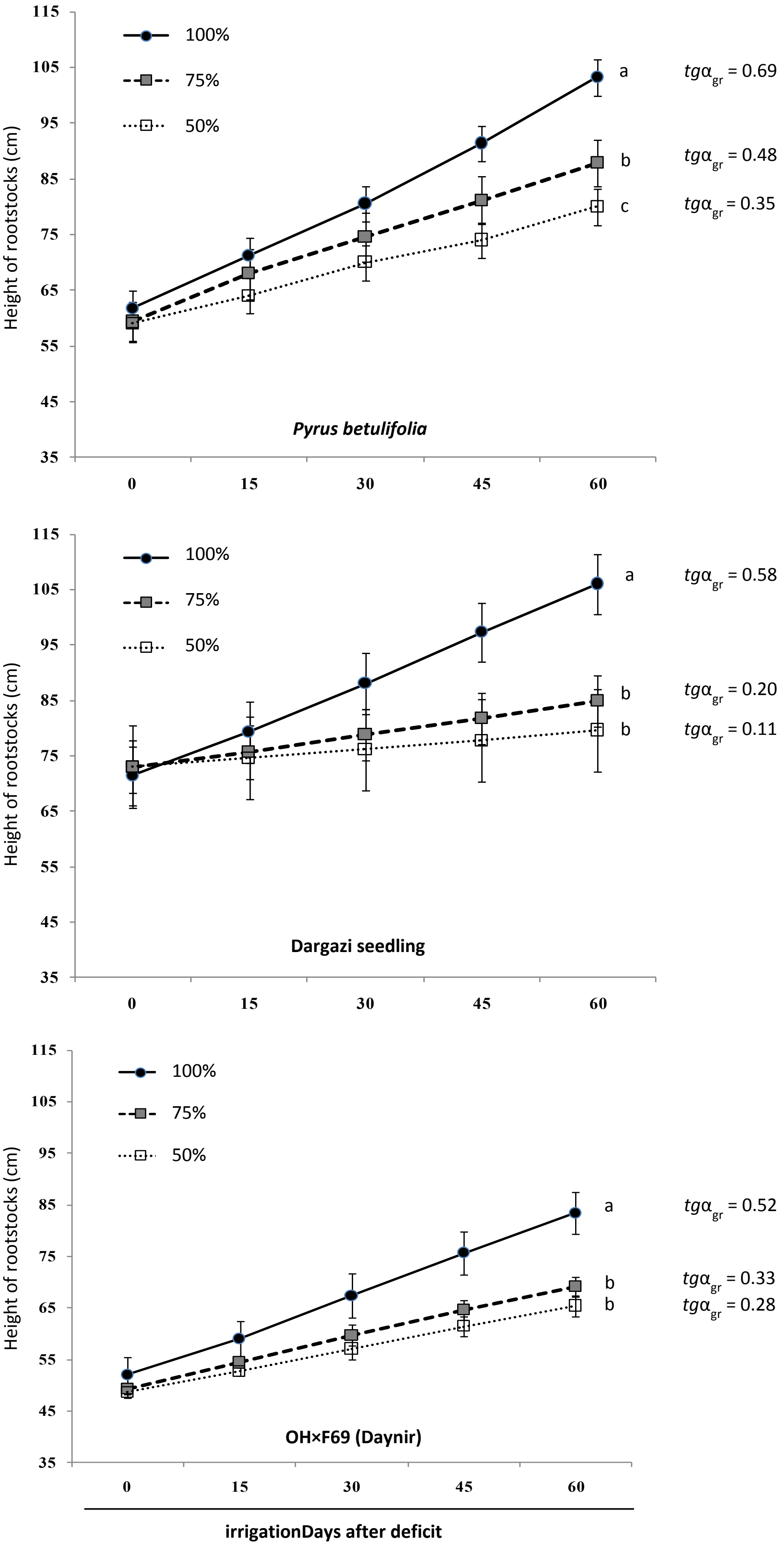
Pear as a key fruit in the temperate areas consumed for its juicy and enjoyable flavor worldly. Like others plant, the growth of pear tree depends on many internal (genetic) and environmental aspects (temperature, moisture availability, different stress, mineral nutrients and etc.) [Wu et al. 2018]. Among the above cases, water restriction is the main aspect of restrictive plant growth and production in various zones of the world [Siddique et al. 2016]. According to the findings of Wu et al. [2013] in pear, vegetative growth is influenced by water shortage thereby diminishing the tree height.

Growth in plants is a permanent increase in size, FW and also DW, which contains cell division, elongation, and differentiation phases. Cell division, development and extension are damaged under various DIR situations (as drought stress). This might be due to the decreasing of water absorption, energy production, unbalanced enzyme activities, turgor pressure, and the loss of energy production. Finally, these cases greatly reduced numerous plant growth and development characteristics such as plant height. Cheng et al. [2012] and Sun et al. [2016] reported that DIR situations significantly influenced growth, water potential and vegetative parameters of pear trees which are in agreement with our results.

Furthermore, RWC, plant and cell water potential, osmotic and pressure potential are the main traits of plant water relatives, which are highly influenced by water shortage owing to a declining in water availability. However, various cultivars and/or species behaved differently; drought-endurance species retained more LRWC for a longer time and wilted later than susceptible species after exposure to water deprivation [Farooq et al. 2012, Ahmad 2016]. Similar to our results Ikinci et al. [2014] mentioned that the different rootstocks had various tolerances to water shortage conditions.

Our study mentioned that TCC enhanced under various DIR in all rootstocks (Fig. 2). The rise in TCC amount with declining of water availability previously mentioned by Giuliani et al. [2018] can be described by a diminution in water content by the fruit without any observable changes in the stored TCC and sugars. Carbohydrates have a fundamental role to provide energy and also compatible solutes (osmoprotectants) in plant organs. Under water shortage situations, plants assemble carbohydrates for example fructans as storing materials that can be mobilized during stages of restricted energy supply or higher energetic requests. Additionally, the rise of carbohydrates have a notable role in the stabilization of various organelle membranes and proteins, maintenance of water absorption, photosynthetic performance, and consequently enhance the plant endurance to water shortage [Sharma et al. 2014, Singh et al. 2015]. Azevedo Neto et al. [2010] reported that under water deficit situations the TCC of leaves and roots significantly increased in tolerant peanut genotypes, which are in coincident with our results.

Furthermore, plants species acclimate themselves to water deprivation by dissipating the surplus stimulation energy thermally with the down-regulation of PSII processes to keep photosynthetic components from photo-damaging impact under water deprivation often simultaneous with increasing of leaf temperature.


115
105
95
85
75
65
55
45
35
Height of rootstocks (cm)
100%
75%
50%
a
b
c
$tg\alpha_{gr}$ = 0.69
$tg\alpha_{gr}$ = 0.48
$tg\alpha_{gr}$ = 0.35
Pyrus betulifolia
0
15
30
45
60
115
105
95
85
75
65
55
45
35
Height of rootstocks (cm)
100%
75%
50%
a
b
b
$tg\alpha_{gr}$ = 0.58
$tg\alpha_{gr}$ = 0.20
$tg\alpha_{gr}$ = 0.11
Dargazi seedling
0
15
30
45
60
115
105
95
85
75
65
55
45
35
Height of rootstocks (cm)
100%
75%
50%
a
b
b
$tg\alpha_{gr}$ = 0.52
$tg\alpha_{gr}$ = 0.33
$tg\alpha_{gr}$ = 0.28
OH×F69 (Daynir)
0
15
30
45
60
irrigationDays after deficit

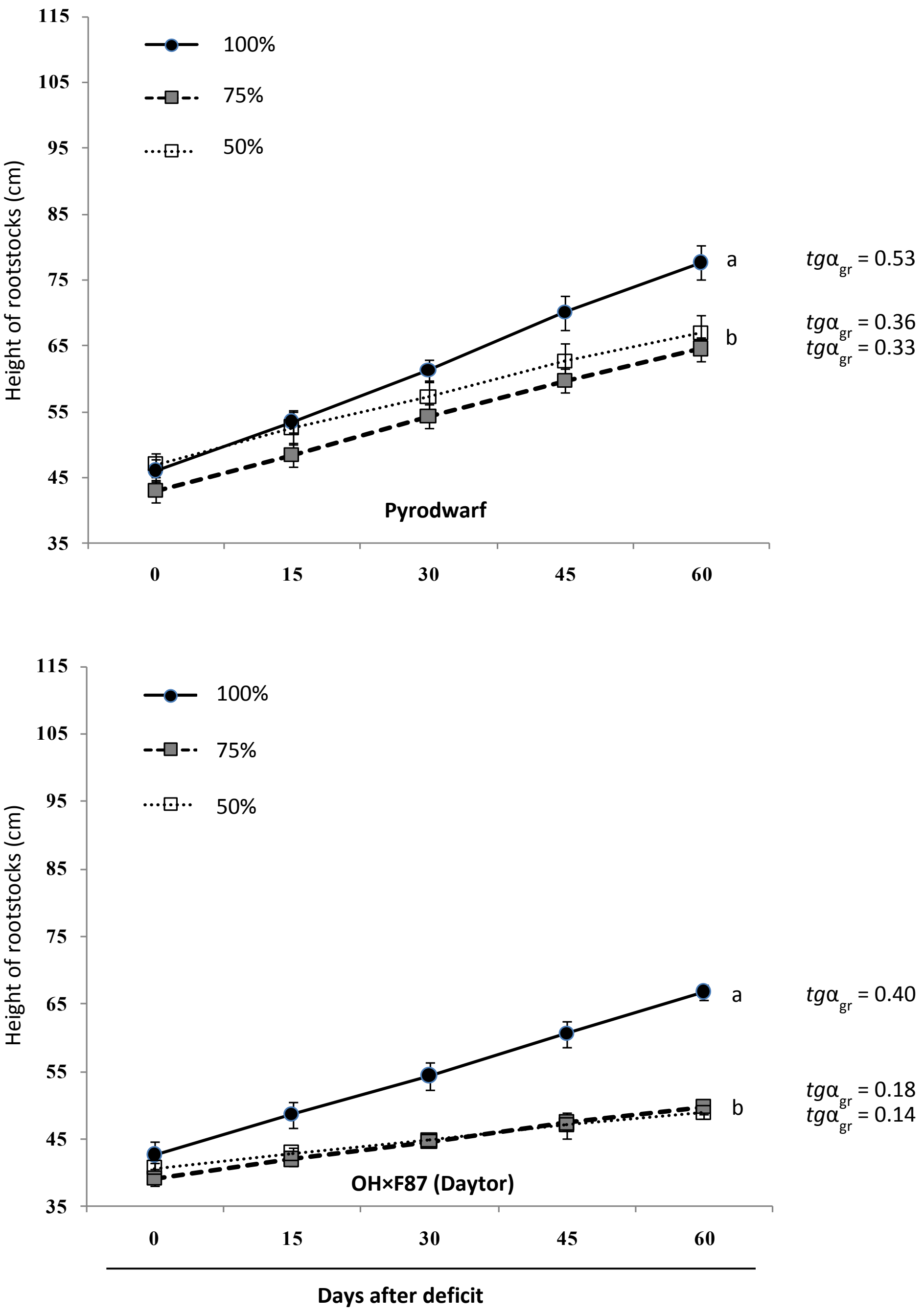


Fig. 1. Comparison the rootstocks growth rates (RGR) of different clonal semi-dwarfing and seedling vigorous pear (*Pyrus* spp.) under various DIR during 60 days. Means of nine replicates with the same letters were not significant differences according to Duncan's multiple range test ($P < 0.01$)

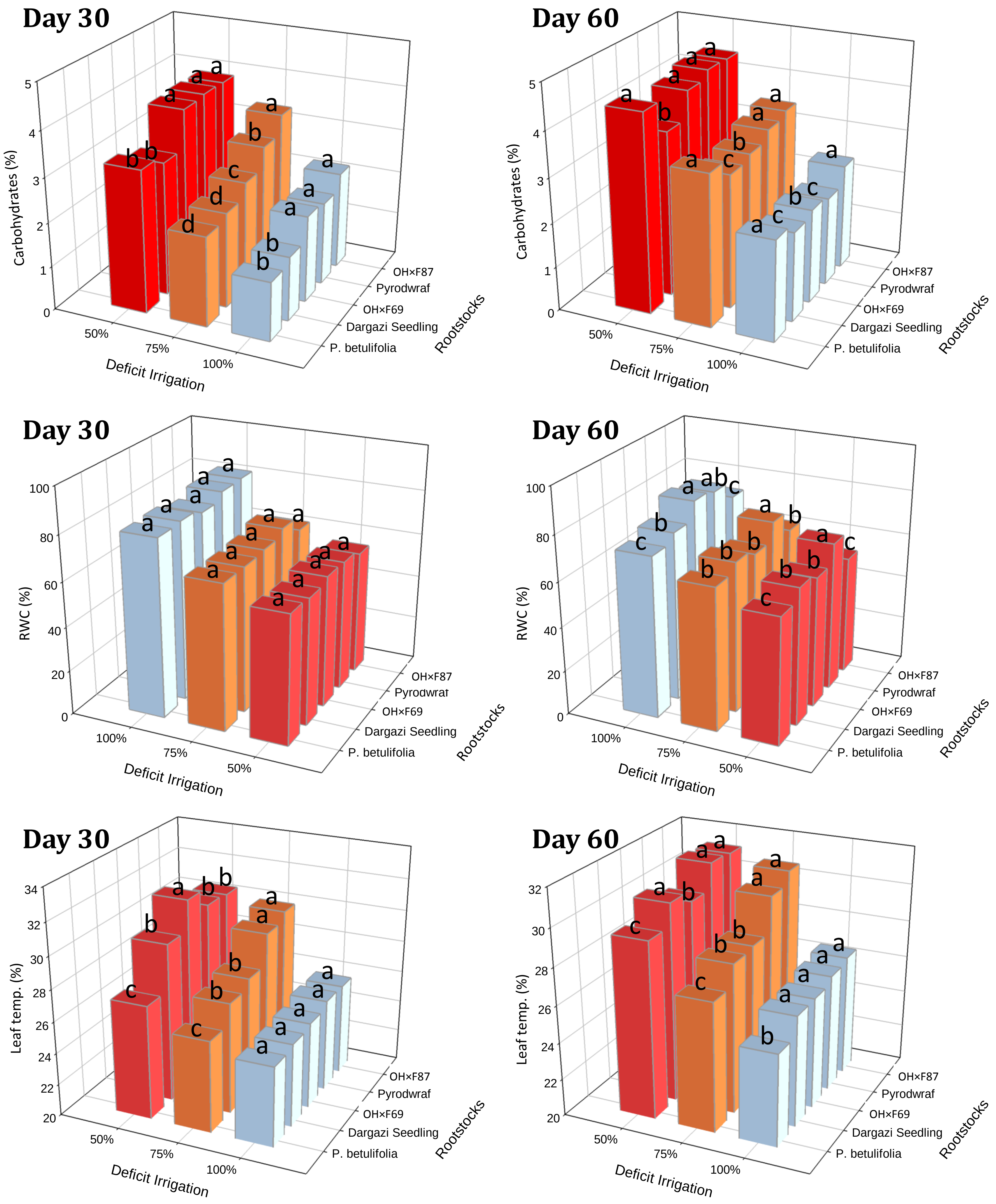


Fig. 2. Comparison the total carbohydrates content (TCC) – upper row – leaf relative water content (LRWC) – middle row – and leaf temperature (lower row) of different clonal semi-dwarfing and seedling vigorous pear (*Pyrus* spp.) under various DIR at two evaluated times (30 and 60 days). Means of nine replicates with the same letters were not significant differences according to Duncan's multiple range test ($P < 0.01$)

Table 1. The simple effects of different pear rootstocks and various DIR on the TCC, LRWC and leaf temperature at two evaluated times

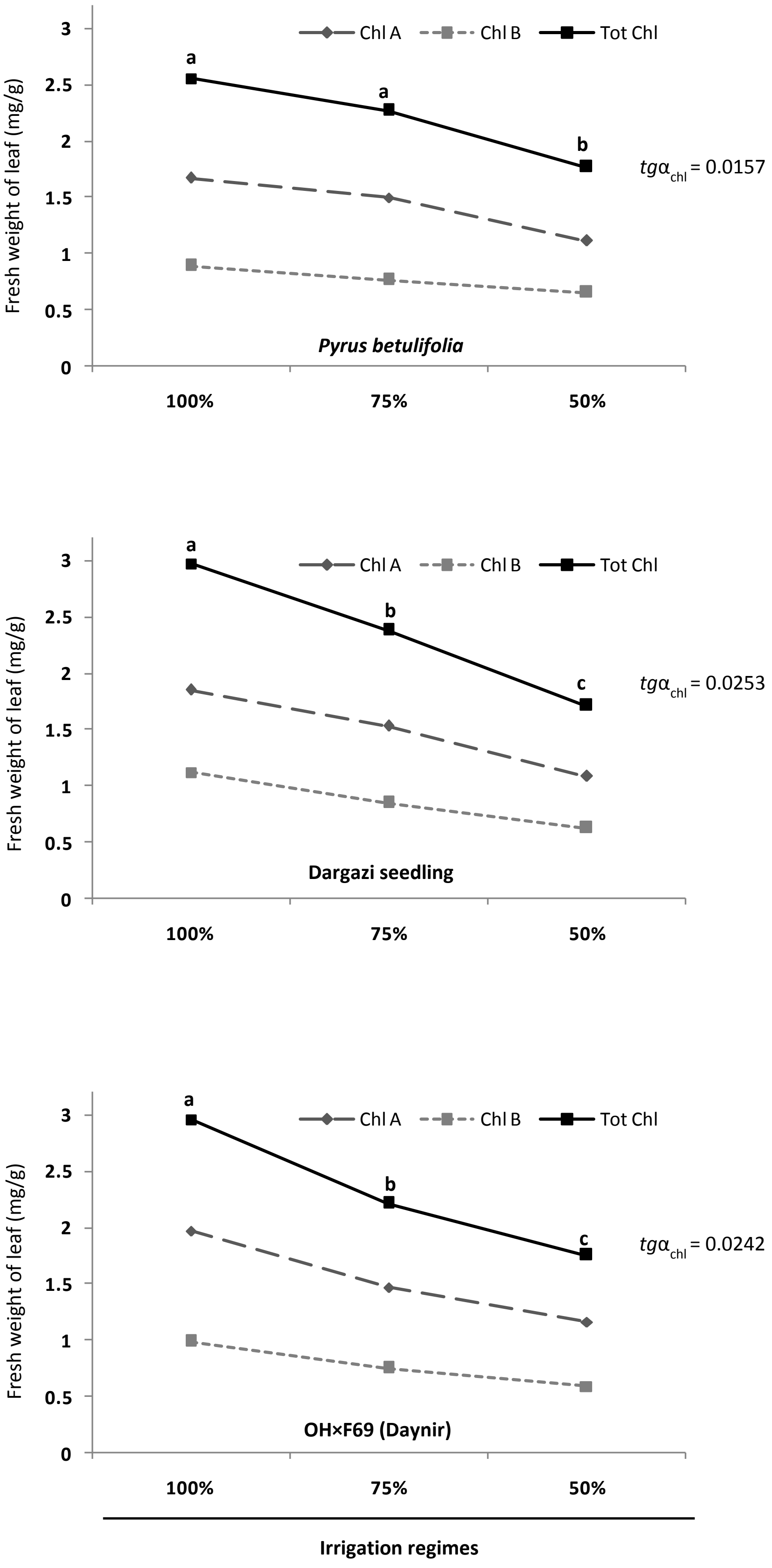
	TCC (%)		LRWC (%)		Leaf temperature (°C)	
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60
Rootstocks						
Pyrus betulifolia	2.21 c	3.38 ab	64.52 c	68.04 a	26.01 b	27.76 c
Dargazi Seedling	2.23 c	2.93 c	68.43 b	67.93 a	28.65 a	29.28 a
OH×F69 (Daynir)	2.79 b	3.20 b	68.90 b	68.07 a	27.42 ab	28.26 b
Pyrodwarf	2.93 b	3.29 b	73.61 a	70.01 a	28.27 a	28.10 b
OH×F87 (Daytor)	3.17 a	3.45 a	63.14 c	66.85 a	28.77 a	29.53 a
Various DIR						
Control (100% of FC)	1.78 c	2.18 c	77.27 a	80.54 a	25.48 c	26.16 c
MDIR (75% of FC)	2.60 b	3.29 b	66.21 b	65.98 b	27.97 b	29.04 b
SDIR (50% of FC)	3.61 a	4.28 a	59.68 c	58.02 c	30.03 a	30.56 a

The same letters on mean values of each column represent not significant differences according to the Duncan's multiple range test ($P < 0.01$). The values are means of nine individual replications

Table 2. The simple effects of different pear rootstocks and various DIR on some photosynthetic parameters after 60 days

	Sub-stomatal CO_2 (C_i)	Transpiration rate (E)	Stomatal conductance (g_s)	Photosynthesis rate (P_N)	WUE (P_N/E)	WUEi (P_N/g_s)
Unit	μmol mol^{-1}	mmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	μmol m^{-2} s^{-1}	μmol mmol^{-1}	μmol mol^{-1}
Rootstocks						
Pyrus betulifolia	296.93 a	4.71 c	0.34 a	14.02 a	2.97 a	43.36 b
Dargazi Seedling	279.89 d	5.57 a	0.25 b	12.40 b	2.20 c	55.89 a
OH×F69 (Daynir)	284.07 c	5.16 b	0.31 ab	13.70 ab	2.63 b	46.99 b
Pyrodwarf	295.15 a	5.30 b	0.34 a	14.00 a	2.64 b	42.98 b
OH×F87 (Daytor)	291.93 b	5.23 b	0.24 b	11.78 c	2.22 c	53.93 a
Various DIR						
Control (100% of FC)	312.11 a	5.80 a	0.45 a	16.85 a	2.92 a	38.47 c
MDIR (75% of FC)	286.22 b	5.14 b	0.26 b	12.86 b	2.53 b	51.49 b
SDIR (50% of FC)	270.44 c	4.64 c	0.19 c	9.83 c	2.15 c	55.94 a

The same letters on mean values of the leaf photosynthetic parameters represent not significant differences according to the Duncan's multiple range test ($P < 0.01$). The values are means of nine individual replications


Chl A
Chl B
Tot Chl
Fresh weight of leaf (mg/g)
0
0.5
1
1.5
2
2.5
3
a
a
b
tgα_chl = 0.0157
Pyrus betulifolia
100%
75%
50%
a
b
c
tgα_chl = 0.0253
Dargazi seedling
a
b
c
tgα_chl = 0.0242
OH×F69 (Daynir)
Irrigation regimes

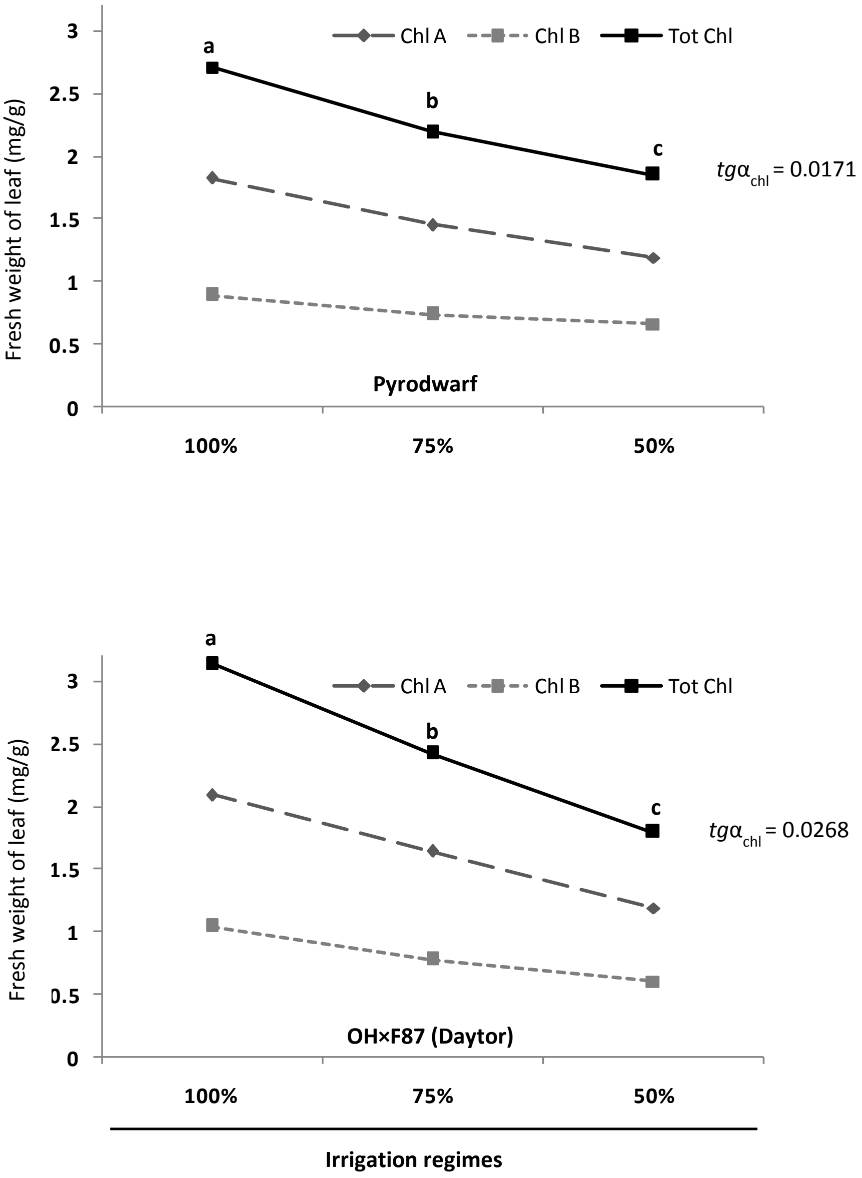


Fig. 3. Comparison the chlorophyll *a*, *b* and total chlorophyll content of different clonal semi-dwarfing and seedling vigorous pear (*Pyrus* spp.) under various DIR during 60 days. Means of nine replicates with the same letters were not significant differences according to Duncan's multiple range test ($P < 0.01$)

Rise in TL might have activated the xanthophyll pigments cycle to safely remove the surplus incitement energy as heat [Shahenshah and Isoda 2010]. Similar to our results, Shahenshah and Isoda [2010] mentioned that water stress significantly enhanced leaf temperature in different cultivars of cotton and peanut.

Under water shortage situations, plants immediately close the stoma to diminish the leaf transpiration, to avoid extra water scarcity in its tissues, which cause inhibition of CO_2 entrance into the leaves. It was revealed that along with stomata closed, leaf temperature enhanced in response to the leave CO_2 input level reduced by less RWC during dryness [Shanker and Venkateswarlu 2011].

The understanding of the physiological processes of plants under dryness situations from the evaluation of chlorophylls content (as a key part of chloroplast for photosynthesis) is essential to developing selecting and breeding programs. It was concluded that drought stress reduced photosynthesis pigment such as chlorophylls content [Farooq et al. 2012]. During water limited situations, the reactive oxygen species (ROS) are created and resulting oxidative stress in the plant. The diminution in chlorophylls amount has been confirmed as a general indicator of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll destruction. Furthermore, chlorophyll degradation during water scarcity might be related to declining in the lamellar amount of chlorophylls. Tolerant species showed the lower reduction of chlorophylls concentration compared to sensitive species [Ahmad 2016, Arabshahi and Mobasser 2017]. Similar to our results, Arji and Arzani [2008] and Guerfel et al. [2009] mentioned that drought stress negatively affected chlorophylls content of different olive cultivars.

As mentioned above, plants control their daily water condition at an appropriate amount by the regulating of stomatal opening. Stomatal closing helps to preserve great leaves RWC and leaves water potential, but it causes a diminution in leaf photosynthesis level. Stomatal closing declines intercellular CO_2 amount in leaves which inflicts restrictions CO_2 absorption, and consequently causes an imbalance among photochemical activity at PSII and electron transference for photosynthesis, and finally resulting in vulnerability to photo-damage. On the other side, diminished CO_2 transmission from the atmosphere to the place of carboxylation is mostly recognized as the chief reason for dwindled photosynthesis under water restriction. Additionally, the influence of abiotic stress on photosynthesis system is different, and not only straightly resulted in photosynthetic system damage but also influenced photosynthetic electron allocation, photosynthetic phosphorylation and enzymes attributed to the photosynthetic dark processes [Ohashi et al. 2006, Pinheiro and Chaves 2011].

Studies mentioned that dryness could obstacle the incoming of CO_2 into the leaves; impact the absorption of CO_2 by the carboxylation system and resulting in the reduction P_N. Generally, P_N declines owing to the stomatal and/or non-stomatal restriction reasons. P_N declines by modification or closing the stoma in stomatal restriction and the photosynthetic cells integrity; organelles defects in non-stomatal limitation [Farooq et al. 2012]. The changes in P_N and g_s amounts are commonly utilized as drought endurance indices in plants. The inhabitation of g_s under water shortage might be owing to the notable reduction in the internal to atmospheric CO_2 amount ratio. Moreover, a dwindle in P_N and g_s is related to an electron transportation level/P_N ratio rise that is explained as indicative a heavy and prevailing role of diminished g_s in photosynthetic down-controlling [Ramalho et al. 2013]. A positive relationship also obtained between P_N and g_s in our study (Fig. 4 C and D). There are several feasible reasons for this correlation under dryness conditions. For example, the alteration in P_N might be mostly a result of variations in g_s, caused by regulations in stomatal part. Likewise, P_N and g_s perhaps co-controlled under water availability restrictions [Romero et al. 2004].

The management of stomata closing and/or opening is vital for plants to control carbon absorption and water deprivation by transpiration. Dwindled transpiration as a result of controlling system to decrease g_s during dryness situations may be a compatibility method to preserve the physiological processes against hydraulic defect during limiting the CO_2 entrance [Brodribb and Mcadam 2011, Mcdowell 2011]. Similar to our results, Jie et al. [2008] reported the effect of drought stress on LRWC, *E*, C_i, g_s and P_N of apricot tree. Moreover, Behboudian et al. [1994] concluded that water deficit significantly influenced the photosynthesis parameters in Asian pear.

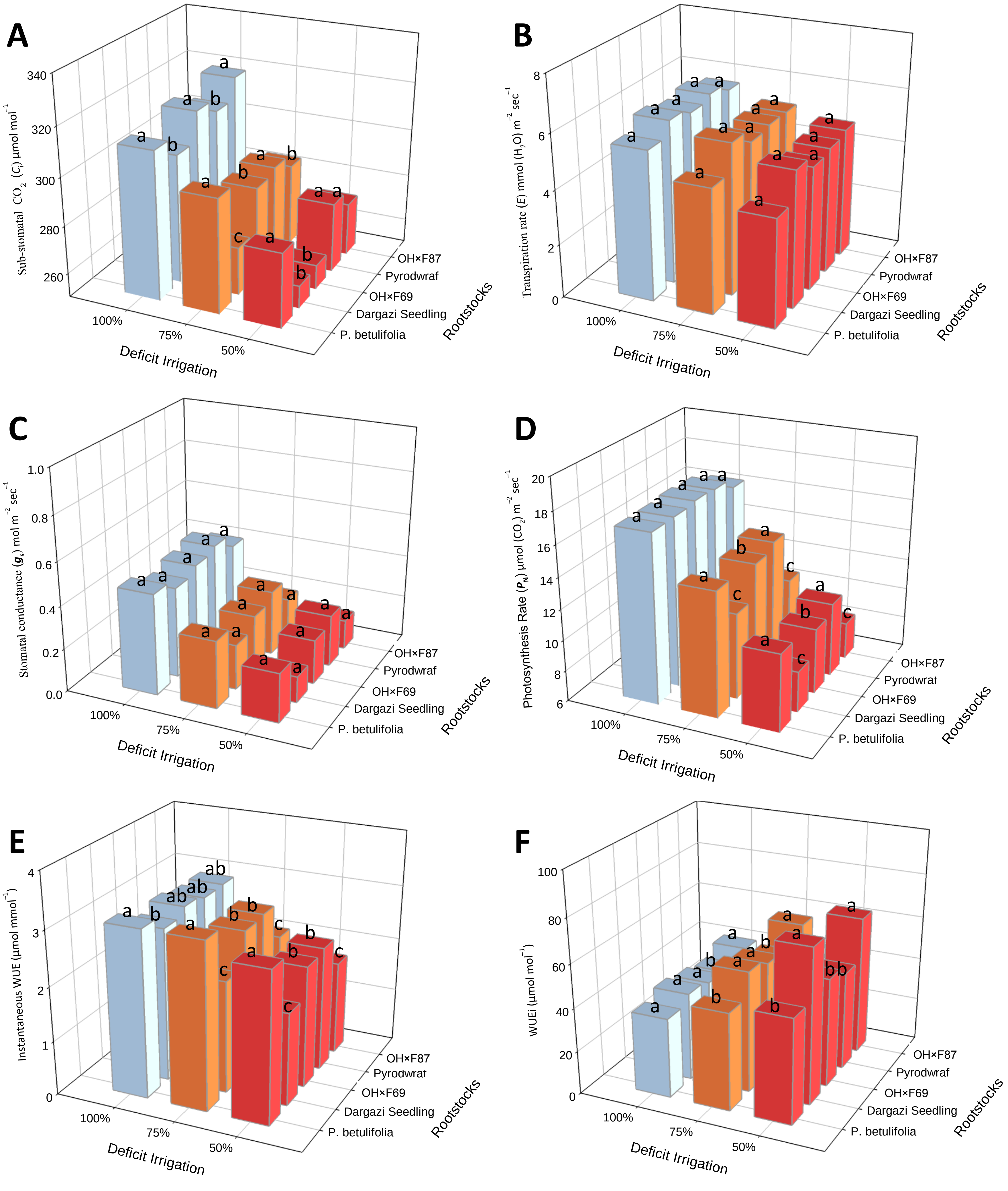


Fig. 4. Comparison the sub-stomatal CO2 (C_i) (**A**), transpiration rate (E) (**B**), stomatal conductance (g_s) (**C**), photosynthesis rate (P_N) (**D**), instantaneous WUE (P_N/E) (**E**) and WUEi (intrinsic WUE) (P_N/g_s) (**F**) of different clonal semi-dwarfing and seedling vigorous pear (*Pyrus* spp.) under various DIR after 60 days. Means of nine replicates with the same letters were not significant differences according to Duncan's multiple range test ($P < 0.01$)

Additionally, a diminish in the g_s of plants declined an already E to evade more water loss and dryness. Under this situation and also closed stomatal, C_i significantly reduced owing to the lower CO_2 entrance [Ahmad 2016, Zhang et al. 2018]. The modification in P_N and E regulated by g_s caused variation in WUE under water deprivation. The decrease in P_N, E, and g_s and rise of WUE under dryness in different cultivars is ordinary [Gago et al. 2014, Zhang et al. 2018]. Hypothetically, WUE may be enhanced by partial stomata closing then the intercellular CO_2 amount is adequate for saturation of P_N while the E can be greatly reduced under slight water deprivation [Liu et al. 2005].

Since an improved WUE can ameliorate plant growth characteristics, WUE is a basic physiological property for selection endurance species under water shortage. WUEi enhanced directly with increasing DIR severity, consequently assistance to preserve high photosynthetic level during various DIR. On the other side, instantaneous WUE diminished under water deprivation because E was fewer influenced by drought than P_N (Fig. 4 C and D). Similar patterns already observed by Romero et al. [2004] in almond trees.

On the other side, our experiment revealed that studied rootstocks (clonal semi-dwarfing and vigorous seedling pear) showed different behavior under normal (well-watered situations) and also various DIR. These results are coincident with Losciale et al. [2008] and Bosa et al. [2016] about photosynthetic traits in various pear cultivars.

Among many aspects associated to drought tolerance in plants, 2 approaches may then be confirmed: a) dehydration avoidance (or postponement) and b) dehydration tolerance. Dehydration avoidance is recognized by solute accumulation (such as osmoprotectants) and cell wall hardening to diminution water deprivation. Dehydration tolerance contains the creation of protective compounds and proteins, physiologic and/or metabolic alterations and ROS elimination to elude injuries created by drought [Santana-Vieira et al. 2016]. In our findings, the *P. betulifolia* and Pyrodwarf rootstocks were detected to display various approaches to tolerate/endurance DIR as drought stress. These findings are in coincident with the findings of Stern et al. [2013] and Tamura [2012].

CONCLUSION

As a conclusion, in all studied species, MDIR and SDIR had an adverse impact on the growth, chlorophylls content, C_i, g_s, P_N and WUE. On the other side, it was revealed that *P. betulifolia* and Pyrodwarf rootstocks showed the better RGR, chlorophylls content, TCC, LRWC C_i, g_s, P_N and instantaneous WUE. Finally, the use of *P. betulifolia* and Pyrodwarf (as drought tolerance rootstocks) could be more suitable under dryness situations.

ACKNOWLEDGMENTS

This research was a collaboration project between Iranian Ministry of Agriculture, Horticultural Sciences Research Institute and the Azad University of Tehran, Science and Research Unit. The authors sincerely thank all technical staff of fruit tissue culture and biotechnology laboratory of Temperate Fruits Research Center of Horticultural Sciences Research Institute for their kind supports.

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