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Effect of lime concentration on pear's rootstock/scion combinations

Akbar Esmaeili[1], *Hamid Abdollahi*[2]*, *Masoud Bazgir*[3], *Vahid Abdossi*[1]

[1]*Department of Horticulture Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Temperate Fruits Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*
[3]*Department of Soil and Water Engineering, Faculty of Agriculture, Ilam University, Ilam, Iran*

**Corresponding author: habdollahii@yahoo.com*

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Abstract: The aim of the research was the determination of the tolerance of different pear grafting combinations to various levels o lime concentration in calcareous soils. The experiment was carried out under pot conditions for a two-year period (2015–2016). Two factors including combination of rootstocks/scions (3 × 3) and levels of lime concentration were employed to evaluate leaf responses including total iron (Fe), available Fe, chlorophyll (Chl) (*a*, *b*, total), and chlorophyll fluorescence attributes (*F*0, *Fm*, *Fv*/*Fm*) as well as annual growth and internode length of current shoots. Results showed that soil lime significantly reduced tree growth but conversely intensified iron chlorosis. Also, contrary to our expectations, the Pyrodwarf/*Pyrus communis* L. (Dargazi) combination displayed more tolerance to high lime concentrations, whereas the OH × F/Williams Duchesse combination did not exhibit suitable tolerance. As a pear rootstock native to Iran, Dargazi seedling rootstock in combination with different scions was found to have relatively better growth under low lime concentrations, but its response under high lime was not as favourable as expected. Cultivar Dargazi combined with different rootstocks showed a better response to high lime stress compared to other scions, while cultivar Williams Duchesse exhibited lower tolerance to high lime concentration when combined with different pear rootstocks.

Keywords: pear; rootstock scion combination; lime; tolerance

Pear (*Pyrus* spp.) is one of the main fruit crops (FAO 2014) extensively grown in the regions with Mediterranean climates (including major parts of Asia) characterised by calcareous soils and low level of annual precipitation (Ma et al. 2005). In these regions, soils contain high bicarbonate ions (Mengel 1994) leading to high pH and low iron (Fe) availability, thereby resulting in the development of lime-induced chlorosis (Pestana et al. 2005; Fraga et al. 2012). Previous studies have shown that in comparison to other temperate fruits, pear is more susceptible to lime-induced Fe chlorosis in a sense that it easily shows signs of severe Fe deficiency (Sanz et al. 1992; Ikinci et al. 2014). The previous studies have demonstrated a negative relationship between Fe deficiency and available Fe in soils depending on bicarbonate and high pH in a way that whenever available Fe in soil due to bicarbonate and high pH is diminished, Fe deficiency occurs and its effects on plants have been meticulously investigated so far (Incesu et al. 2015).

As a major nutritional problem of fruit trees growing on calcareous soils, Fe chlorosis is closely associated with Fe deficiency and related reduction in photosynthetic pigments (Pestana et al. 2005). Regarding the vital role of Fe in plant health under stressful conditions, the introduction of proper approaches to mitigate Fe deficiency has attracted researchers and breeders (Tagliavini, Rombola 2001). Using iron fertilizers in forms of spray or soil mixtures has been introduced by authors, but these approaches are costly and lead to natural resources' pollution. Accordingly, application of proper rootstocks individually or in combination with suitable scions under calcareous conditions is a simple and reliable approach that has received great attention (Ma et al. 2005; Alcantara et al. 2003; Mestre et al. 2017).

As rootstocks are the main controllers of several characteristics such as mineral uptake and tree growth and also confer their appropriate traits to scions (Hanana et al. 2015; Barazani et al. 2017), employing appropriate rootstocks grafted with suitable scions is imperative to induce Fe chlorosis tolerance (Tagliavini et al. 1995; Sotiropoulos 2006). Also, it has been proved that different rootstocks show different responses to stressful conditions (Sugar, Basile 2011). Although the role of rootstocks on Fe chlorosis attenuation under Mediterranean conditions has been extensively investigated (Reig et al. 2016; Mestre et al. 2017), determination of an efficient pear rootstock/scion combination tolerant to calcareous soils has not been meticulously addressed yet. Thus, the main objective of the current research was to determine the tolerance of different pear rootstock/scion (R/S) combinations under different lime concentrations in terms of morphological and physiological variables to mitigate the harmful effects of lime on pear orchards.

Table 1. Means of some variables of pear Rootstock/Scion (R/S) combinations under different lime concentrations located at Ilam experimental station during two years, 2015–2016

Rootst./Scion combination.	Fe total (ppm)	± SE	Fe avl. (ppm)	± SE	Internode length (cm)	± SE	Annual growth (cm)	± SE
L1C1	30.5^{fj}	0.98	9.9^{eg}	0.14	1.31^{i}	0.12	29.1^{e-g}	4.24
L1C2	42.4^{ab}	2.03	13.9^{ab}	0.57	1.43^{i}	0.23	31.3^{de}	3.11
L1C3	29.5^{ij}	0.52	9.5^{hi}	0.2	1.60^{h}	0.26	28.7^{e-g}	1.84
L1C4	31.5^{eg}	0.66	10.2^{ef}	0.17	2.23^{d}	0.22	25.1^{h-j}	2.90
L1C5	44.9^{a}	1.47	14.7^{a}	0.45	3.00^{a}	0.66	37.7^{a}	2.83
L1C6	31^{eh}	0.79	10^{eg}	0.16	2.90^{ab}	0.36	35.1^{a-c}	2.97
L1C7	31.7^{ef}	0.94	10.2^{ef}	0.39	1.57^{h}	0.25	28.7^{e-g}	0.49
L1C8	41^{b}	0.68	13.2^{b}	0.33	2.52^{c}	0.50	36.1^{ab}	1.84
L1C9	29.9^{hj}	0.56	9.7^{gi}	0.19	2.22^{de}	0.22	31.7^{c-e}	1.70
L2C1	25.6^{mn}	0.58	8.3^{kl}	0.2	1.31^{i}	0.21	24.7^{h-j}	2.83
L2C2	32^{e}	0.75	10.3^{e}	0.29	1.27^{i}	0.15	31.3^{d-e}	2.40
L2C3	26.8^{lm}	0.52	8.6^{jk}	0.38	1.60^{h}	0.56	23.1^{i-l}	2.97
L2C4	29^{jk}	0.93	9.4^{i}	0.34	2.13^{d-f}	0.12	27.7^{f-h}	3.75
L2C5	37^{c}	1.95	12^{c}	1	3.10^{a}	0.56	29.1^{e-g}	2.97
L2C6	27.5^{l}	0.26	8.8^{l}	0.28	2.72^{L}	0.47	31.7^{c-e}	5.52
L2C7	27.6^{l}	0.27	8.9^{l}	0.27	1.57^{h}	0.25	22.7^{j-l}	2.83
L2C8	35.5^{d}	0.265	11.5^{d}	1.17	2.00^{e-f}	0.70	33.3^{b-d}	1.84
L2C9	27.5^{l}	0.49	8.8^{l}	0.39	2.21^{de}	0.21	24.3^{h-j}	3.25
L3C1	20.5^{p}	0.68	5.9^{o}	0.36	1.23^{i}	0.21	19.1^{mn}	1.27
L3C2	28.1^{kl}	2.31	8.2^{l}	0.96	1.33^{i}	0.22	23.7^{i-k}	2.83
L3C3	19.2^{p}	2.5	5.6^{o}	0.91	1.40^{i}	0.40	17.1^{n}	1.41
L3C4	25^{n}	2.82	7.3^{m}	0.94	1.93^{fg}	0.40	20.2^{l-n}	4.24
L3C5	30.2^{gj}	2.2	8.8^{l}	0.92	2.80^{c}	0.80	30.0^{d-f}	1.41
L3C6	22^{o}	1.39	6.4^{n}	0.61	2.54^{c}	0.50	21.3^{k-m}	3.75
L3C7	23^{o}	1.75	6.7^{n}	0.74	1.37^{i}	0.35	22.3^{j-m}	1.84
L3C8	27.5^{l}	2.18	8.8^{j}	0.91	1.80^{g}	0.70	26.3^{g-i}	2.83
L3C9	19.6^{p}	2.91	5.7^{o}	1.04	1.96^{fg}	0.57	20.2^{l-n}	1.41

means in each column with the same letters are not significantly different at 1% and 5% level of Duncan multiple range test; L1 – lime10%, L2 – lime15%, L3 – lime 20%; C1 – seedling/Louise Bonne, C2 – seedling/Dargazi, C3 – seedling/Williams Duchesse, C4 – Pyrodwarf/Louise Bonne, C5 – Pyrodwarf/Dargazi, C6 – Pyrodwarf/Williams Duch; C7 – OH × F69/Louise Bonne, C8 – OH × F69/Dargazi, C9 – OH × F69/Williams Duchesse

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MATERIALS AND METHODS

This research was conducted at the Ilam Agricultural and Natural Resources Research Station located in Sarabelah, Ilam, Iran (33°33'N, 46°25'E, and 947.1 m a.s.l.), for a two-year period (2015–2016). In this study, the grafted combinations included three 1-year old rootstocks (Seedling, Pyrodwarf, and OH × F69) and three scions (Louise Bonne, Dargazi, Williams Duchess) in a total of nine combinations encoded as C1 to C9 (Table 1) grown in the same nursery and grafted under identical conditions.

All rootstock/scion combinations were transferred to 20 l pots filled with a mixture of land soil taken at 0–30 cm depth characterized by fine loamy, mixed, typic haploxerepts, and lime stone derivation as well as the physico-chemical properties presented in Table 2. In order to prepare 10%, 15%, and 20% lime concentrations in a 10-kg-soil sample, the amounts of 720, 1,080, and 1,440 mg lime ($CaCO_3$) were added to the soil, respectively. The pots were arranged in a randomized complete block design (RCBD) in three replicates and irrigated with lime-free tap water twice a week as well as identically grown under natural conditions. Moreover, samples were not supplied with any fertilizer but pruned in winter each year. At the end of seasonal growth, in order to measure total Fe, available Fe, and chlorophyll content of leaves, three matured leaves located in the middle of current shoots were taken as a sample per a replicate and immediately transferred into the lab. In this respect, total Fe based on Jones and Case (1990), Fe availability based on Katyal and Sharma (1980), chlorophyll (Chls) content based on Lichtenthaler and Wellburn (1987) variables were measured. To evaluate chlorophyll fluorescence (Chl F), four matured leaves located in the middle of current shoots as a sample per a replicate were selected at ambient temperature (24°C to 26°C) in the morning (at 9 o'clock), and were then wrapped in foil to providing required darkness for 20 min followed by measuring Chl F with a portable fluorometer (PAM-2500; Walz, Effeltrich, Germany). The minimum fluorescence ($F0$) was obtained after exciting Chl with a weak beam of a light-emitting diode. Maximum fluorescence (Fm) was determined by irradiance of a 600-ms pulse of saturated white light. The ratio of Fv/Fm was measured by the equation: $(Fm – F0)/Fm$. Also, annual growth (AG) and internode length (IL) of current shoots as indicators of plants' growth were measured by digital clipper and rule.

The data were initially normalized, and they were then analyzed by a two-way analysis of variance (ANOVA). Differences between means were calculated by using Duncan Multiple Range Test at significance levels of $P \leq 0.01$ and $P \leq 0.05$. To examine the strength of a relationship between two variables, Pearson correlation coefficients were calculated. All data were analyzed using PROC GLM of SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

The analysis of variance showed significant difference for $F0$ and Fv/Fm traits at 5% level, and annual growth, chlorophyll a, chlorophyll b, total chlorophyll, internode Length, total Fe, active Fe at 1% level (Table 3).

The results of this experiment showed that the rates of both Fe types (i.e. total and available) were depressed by an increase in lime (L) concentration of the soil, and accordingly the lowest values of both Fe types were recorded in the C9 (OH × F69/ Williams Duchesse) combination grown in 20% lime. The highest Fe types values in the leaves were observed in the C5 (Pyrodwarf/Dargazi) combination in 10% lime (i.e. control). The values of both Fe types were gradually reduced by increasing lime concentration u to 20% (Table 3).

Table 2. Main physico-chemical properties of the soil used in the research

S.A.R	Mg (mg/l)	Na (mg/l)	Ca (mg/l)	Zn (ppm)	Fe (ppm)	Texture	Clay (%)	T.N.V (%)
0.03	8	0.11	19	0.23	3.2	Silt clay	42	9.87
Silt (%)	**Sand (%)**	**K (ppm)**	**P (ppm)**	**Organic Carbone (%)**	**EC (ds/m)**	**pH (T.N.V10%)**	**pH (T.N.V15%)**	**pH (T.N.V20%)**
48	10	250	9.8	1.2	0.36	7.55	7.79	7.98

S.A.R. – sodium absorption ratio; T.N.V – total neutralizing value

Table 3. ANOVA of some variables of pear rootstock/scion (R/S) combinations under different lime concentrations located at Ilam experimental station during two years, 2015–2016

SOV	DF	Total Fe	Active Fe	Inter-node length	Total Chl	Chl*b*	Chl*a*	Annual growth^1	*Fv*/*Fm*	*Fm*	*F*0
Year	1	27**	24.4**	2.7**	2,653**	1,061**	629**	2,656**	0.09**	3,721**	10,707**
Bblock	4	0.1^{ns}	0.97ns	0.04**	38**	25**	109**	29**	0.003*	1760^{ns}	797**
Llime	2	259**	1,667**	1.1**	41**	0.9^{ns}	43**	1,204**	0.3**	108,103**	27,857**
Lime × Year	2	3.6**	65**	0.2**	6^{ns}	0.7^{ns}	9^{ns}	31^{ns}	0.001^{ns}	131^{ns}	92^{ns}
Rootstock	2	7.3**	77**	19**	14^{ns}	30**	2.6^{ns}	154**	0.004**	2091^{ns}	400^{ns}
Rootstock × Year	2	0.4*	4.6**	0.03^{ns}	15*	13*	11.5*	239*	0.0001^{ns}	279^{ns}	1.7^{ns}
Lime × Rootstock	4	1.3**	13**	0.09**	3^{ns}	1.7^{ns}	1^{ns}	13**	0.002^{ns}	1,214^{ns}	87^{ns}
Lime × Rootstock × Year	4	0.11^{ns}	1.2ns	0.01^{ns}	6^{ns}	1.6^{ns}	3.9^{ns}	20^{ns}	0.00003^{ns}	2,227^{ns}	52^{ns}
Scion	2	152**	1530**	4.4**	114**	78**	10^{ns}	639**	0.006**	18,129**	66^{ns}
Scion × Year	2	1.2**	7*	0.01^{ns}	63**	28**	14*	153**	0.00001^{ns}	3,857^{ns}	99^{ns}
Lime × Scion	4	8.4**	72**	0.01^{ns}	7^{ns}	3^{ns}	1.7^{ns}	53^{ns}	0.005**	4,098^{ns}	446*
Lime × Scion × Year	4	0.3*	3.5*	0.004^{ns}	5^{ns}	0.7^{ns}	3^{ns}	36^{ns}	0.0001**	657^{ns}	68^{ns}
Rootstock × Scion	4	0.8**	7.6**	0.9**	41**	23**	8.2^{ns}	66*	0.006^{ns}	5,051*	580**
Rootstock × Scion × Year	4	0.1^{ns}	1.3ns	0.01^{ns}	13*	1.5*	1.1^{ns}	100**	0.0001^{ns}	1,528^{ns}	129^{ns}
Lime × Scion × Rootstock	8	0.9**	8.4**	0.06**	6.2^{ns}	9.5^{ns}	3.4^{ns}	9.9**	0.002*	5699**	225*
Lime × Rootstock × Scion × Year	8	0.1^{ns}	1.3^{ns}	0.01^{ns}	9.5^{ns}	2^{ns}	4.5^{ns}	14^{ns}	0.0001^{ns}	931^{ns}	68^{ns}
Error	104	0.1	1.1	0.009	4.9	2.9	3.4	1.5	0.001	1,886	138

*, **significant at the 5% and 1% probability levels, respectively; ns – not-significant; ^{1}growth of pear under different lime concentrations; SOV – standard of variation; *Fm* – maximal fluorescence, *F*0 – min. fluorescence;

Lime significantly influenced chlorophylls contents. The findings of this study revealed a negative relationship between L concentration and chlorophyll content. The results of this research highlighted that although the combination of C6 (Pyrodwarf/Williams Duchesse) was found to gain the highest values in terms of Chl*b* and total Fe at 10% lime, it was only the C5 combination obtained the highest values of Chl*b* and total at 20% lime (Table 1). Interestingly, the results obtained by applying an interactional effect of R/S and lime on Chl*a* were different from those recorded by their application on Chl*b* and total, that is, the highest and lowest values of Chl*a* were gained by the C4 (Pyrodwarf/Louise Bonne) combination at 10% lime and the C9 (OH × F69/Williams Duchesse) combination at 20% lime, respectively. Also, no significant difference was observed between the C6 and C5 combinations, although the C6 (Pyrodwarf/Williams Duchesse) combination was found more tolerant at 20% lime in comparison to the C5 combination (Table 4).

The data presented in Table 1 indicate that lime stress can enhance the amount of *F*0, whereas this trend was not observed in *Fm* and *Fv*/*Fm* attributes. Also, this trend manifested that these attributions (i.e. *Fm* and *Fv*/*Fm*) had positive relationship with each other, but they had a negative relationship with *F*0 under stressful conditions (Table 4). The highest value of *F*0 was found in the C8 combination at 20% lime while the lowest ones was observed in the C1 combination (Seedling/Louise Bonne) under 10% lime condition. In contrast, the highest values of *Fm* and *Fv*/*Fm* were found in the C5 combination at 15% lime, but the lowest values were obtained in the C9 combination (OH × F69/Williams Duchesse) at 20% lime (Table 4).

Also, the lime significantly affected the growth habits of pear. In this regard, annual growth and internode length were used as morphological indicators of pear R/S combinations' response to different lime concentrations. In this respect, the C5 combination (Pyrodwarf/Dargazi) significantly showed longer internodes and higher annual growth at different lime concentrations (Tables 1 and 4).

Results in Table 5 suggest that a positive correlation is not only observed between total Fe and

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Table 4. Means of some variables of pear of Rootstock/Scion(R/S) combinations under different lime concentrations located at Ilam experimental station during two years, 2015–2016

Rootstock/ Scion comb.	Chl*a*	± SE	Chl*b*	± SE	Chl total	± SE	*F*0	± SE	*Fm*	± SE	*Fv*/*Fm*	± SE
L1C1	9.61^{a-c}	0.44	5.10^{b-d}	1.56	12.93^{e-g}	2.76	104^{g}	5.66	547^{a-c}	5.66	0.807^{a}	0.42
L1C2	11.20^{a-c}	1.70	5.42^{a-d}	2.01	14.97^{c-g}	2.73	122^{e-g}	2.83	567^{ab}	4.24	0.784^{ab}	0.13
L1C3	9.32^{bc}	1.41	7.40^{a-d}	1.70	15.20^{c-g}	0.28	108^{fg}	2.83	473^{c-h}	5.30	0.772^{a-c}	0.14
L1C4	14.60^{a}	2.83	6.92^{a-d}	1.48	20.71^{ab}	3.68	116^{fg}	7.07	539^{a-d}	2.12	0.792^{ab}	0.29
L1C5	10.50^{a-c}	0.99	8.11^{a-d}	2.98	16.73^{a-f}	2.40	106^{fg}	5.66	528^{a-f}	2.55	0.791^{ab}	0.28
L1C6	14.40^{ab}	3.39	9.20^{a}	1.70	21.20^{a}	3.11	124^{d-g}	2.83	455^{f-h}	3.54	0.728^{b-d}	0.25
L1C7	8.64^{c}	1.41	6.12^{a-d}	0.88	13.22^{d-g}	1.77	108^{fg}	2.12	514^{a-g}	2.47	0.790^{ab}	0.28
L1C8	8.94^{c}	0.71	8.43^{a-c}	0.61	11.72^{fg}	0.99	112^{fg}	1.41	528^{a-f}	3.04	0.784^{ab}	0.28
L1C9	8.63^{c}	1.86	4.00^{d}	0.71	13.92^{d-g}	2.69	112^{fg}	8.49	537^{a-e}	2.40	0.758^{a-c}	0.36
L2C1	12.63^{a-c}	2.87	6.52^{a-d}	0.78	17.32^{a-e}	1.84	120^{fg}	2.83	531^{a-e}	2.90	0.773^{a-c}	0.28
L2C2	9.00^{c}	0.71	5.53^{a-d}	2.11	13.12^{e-g}	2.33	110^{fg}	4.24	531^{a-e}	4.38	0.785^{ab}	0.29
L2C3	12.24^{a-c}	2.88	7.10^{a-d}	1.56	17.32^{a-e}	1.84	119^{fg}	1.41	518^{a-g}	4.31	0.770^{a-c}	0.42
L2C4	8.72^{c}	1.41	7.43^{a-d}	1.77	11.62^{f-g}	2.26	113^{fg}	7.07	535^{a-e}	1.98	0.788^{ab}	0.42
L2C5	10.12^{a-c}	1.06	9.20^{a}	1.13	17.32^{a-e}	1.84	111^{fg}	2.47	587^{a}	1.84	0.809^{a}	0.27
L2C6	8.74^{c}	1.32	7.90^{a-d}	2.16	14.92^{c-g}	2.83	124^{d-g}	1.77	468^{d-h}	4.10	0.717^{c-e}	0.26
L2C7	10.21^{a-c}	1.54	4.13^{d}	0.16	15.81^{c-g}	1.20	128^{c-f}	1.41	506^{b-g}	3.32	0.780^{ab}	0.31
L2C8	9.13^{c}	2.20	8.65^{ab}	1.77	15.91^{b-g}	2.69	112^{fg}	4.24	549^{a-c}	2.90	0.796^{ab}	0.57
L2C9	12.91^{a-c}	2.76	8.62^{ab}	2.05	19.52^{a-c}	2.12	118^{fg}	3.54	489^{c-h}	4.31	0.755^{a-c}	0.35
L3C1	8.94^{c}	2.76	4.41^{cd}	1.63	12.11^{fg}	1.56	167^{a}	4.24	414^{h}	3.54	0.797^{g}	0.29
L3C2	11.52^{a-c}	0.85	6.21^{a-d}	1.57	15.91^{b-g}	1.41	161^{ab}	2.83	448^{gh}	2.90	0.641^{fg}	0.51
L3C3	8.73^{c}	1.44	7.20^{a-d}	1.70	14.32^{d-g}	1.84	145^{a-c}	8.49	461^{e-h}	2.90	0.686^{d-f}	0.44
L3C4	10.12^{a-c}	0.17	5.31^{a-d}	1.84	13.82^{d-g}	1.48	152^{ac}	3.54	426^{h}	2.97	0.643^{f-g}	0.50
L3C5	11.20^{a-c}	2.55	8.82^{ab}	2.47	18.31^{a-d}	2.40	142^{b-e}	4.24	473^{c-g}	1.41	0.686^{d-f}	0.45
L3C6	11.34^{a-c}	3.31	9.10^{a}	1.56	14.70^{c-g}	1.41	145^{a-d}	3.54	443^{gh}	4.74	0.628^{fg}	0.49
L3C7	7.84^{c}	0.25	4.81^{b-d}	2.55	11.52^{g}	1.41	149^{a-c}	4.24	449^{gh}	2.12	0.657^{e-g}	0.49
L3C8	10.91^{a-c}	2.83	8.00^{a-d}	1.41	17.72^{a-e}	1.48	165^{a}	4.24	443^{gh}	3.89	0.671^{d-f}	0.46
L3C9	7.82^{c}	1.98	6.21^{a-d}	1.71	12.62^{e-g}	1.48	157^{ab}	5.44	414^{gh}	2.97	0.646^{fg}	0.50

for explanations see Table 1

Fe availability but also between both Fe types and Chl*b*, total, annual growth, internode length, *Fm*, and *Fv*/*Fm* parameters. On the contrary, it was found a negative relationship between both Fe types and *F*0.

DISCUSSION

Presence of calcium carbonate ($CaCO_3$), as the lime source in this research, can impair uptake and absorption of some nutrients like Fe. However, plants' sensitivity to lime will differ depending on some factors such as type of species experiencing lime stress. The results of this experiment showed that the rates of total and available Fe types in leaf tissue could be affected not only by the rootstock/scion combinations but also by lime concentration in soil. In this respect, the C5 combination (Pyrodwarf/Dargazi) clearly alleviated the adverse effects of high L concentrations (15% and 20%) in soils through saving available Fe content in plants tissues. The findings of this experiment are in agreement with those by Bavaresco and Lovisolo (2000), Sotomayor et al. (2014) and Covarrubias et al. (2016) who highlighted the role of rootstocks and their combinations with scions in mitigating leaf chlorosis caused by Fe deficiency. Furthermore, the results of this research confirmed that rootstocks usually confer their appropriate

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Table 5. Correlation between chlorophyll fluorescence attributes and physiological traits of pear located at Ilam experimental station during location two years, 2015–2016

Treatment	AG	IL	Chl*a*	Chl*b*	Chl total	*F*0	*Fm*	*Fv*/*Fm*	*Fe* aver..
IL	0.22514**	*							
Chl*a*	–0.08412^{ns}	0.18861*							
Chl*b*	0.19823**	0.43533**	0.4405**	*					
Chl total	0.22651**	0.36903**	0.84539**	0.85192**	*				
*F*0	–0.54784**	0.00784^{ns}	0.01899^{ns}	0.17722**	–0.17601*				
Fm	0.4315**	–0.00889^{ns}	–0.03525^{ns}	0.20052*	0.13986^{ns}	–0.51413**			
Fv/*Fm*	0.55792**	0.01798^{ns}	0.00469^{ns}	0.24613**	0.14371*	–0.92502**	0.77178**		
Fe available	0.70696**	0.30006**	0.0867^{ns}	0.04856^{ns}	0.02168^{ns}	–0.60717**	0.56875**	0.68112**	
Fe total	0.64699**	0.33959**	0.14862^{ns}	0.04089^{ns}	0.11102^{ns}	–0.50783**	0.49868**	0.5839**	0.98399**

*, **significant at the 5% and 1% probability levels, respectively; ns – not significant; AG – annual growth annual growth of current shoots; IL – internode length of current shoots;

genetic traits to the scions for elevating tree tolerance to abiotic stresses such as drought and soil pH as previously proposed by Hanana et al. (2015), Meggio et al. (2014) and Barazani et al. (2017).

The results of this research also demonstrated that OH × F69 rootstock in comparison to the other two rootstocks showed inappropriate response to tolerate lime stress, and accordingly employing this rootstock on regions with high lime cannot be recommended. As both OH × F69 and Pyrodwarf are dwarfing rootstocks, the reason of superiority Pyrodwarf over OH × F69 in term of their tolerance to calcareous soils needs further investigation.

Overall, in order to alleviate Fe deficiency, plants exploit two strategies (Tagliavini, Rombola 2001). At first strategy which employed by dicots such as pears, plants embark to secrete H$^+$ through their roots; and such response to Fe deficiency helps plants to decrease the rate of pH in soil but increase Fe solubility. In second strategy which used by grasses, plants secrete the siderophores to be combined with Fe^{3+} in favor of improving plants' accessibility to Fe in soils. It is worth noting that the rate of pH in soil as well as in apoplasm profoundly influences Fe uptake (Alcantara et al. 2003). Secretion H$^+$ into apoplasm facilitates Fe solubility and uptake in plants, but sometimes H$^+$ is improperly secreted into rhizosphere and become inappropriate to improve Fe uptake under calcareous soil. The uptake of Fe is occurred within roots and leaves' mesophylls, and the mechanisms, by which Fe is translocated across plasma membrane, in the both organs are the same. In mechanism of Fe uptake, Fe^{3+} reductase enzyme and Fe^{2+} channel are vital. Fe^{3+}reductase is an enzyme responsible for crossing Fe from the membranes adhered in one side to apoplasm and in other side to cytosol. In this regard, the role of cytosol on supplying the required energy (NADPH) for reductase enzyme activity during reducing Fe^{3+} to Fe^{2+} is also crucial; and this enzyme is so sensitive to apoplasmic pH that an increase in such pH caused to a reduction in enzyme activity (Zribi 2002). Some factors, such as presence of HCO$_3^-$ ion in plants as well as to feed plants with fertilizers containing NO$_3$ ion, profoundly decrease Fe translocations in plants through elevating pH inside plants. These factors generally consume large amount of energy involved in uptake and translocation of Fe and other plant nutrients instead of being used in other essential metabolisms of plants; and accordingly the growth of such plants is reduced (Marschner 2012).

The results of this research confirmed the abovementioned findings in a way that the growth of pears, regardless of type of rootstocks/scions combination, was reduced by increasing lime concentration. A reduction in plant growth under calcareous soils has been reported in findings of Sotomayor et al. (2014) which are in agreement with ours.

In order to detect Fe deficiency in plants, numerous methods such as content of chlorophyll, rate of photosynthesis efficiency, and color of leaves have been introduced so far (Alcantara et al. 2000; Incesu et al. 2015; Covarrubias et al. 2016). Visual symptoms are the shortest and simplest but unreliable ways to detect Fe deficiency in plants (Marschner 2012). The visual symptoms of Fe deficiency in plants, depending on stage at which they experience Fe deficiency, are various. For ex-

Fig. 1. Iron–chlorosis process during leaf growth and development. A new born leaf (left; no symptom of chlorosis) and a mature pear leaf (right; exhibiting chlorosis symptom)

ample, the size of Fe-deficient leaves at initial stage remains constantly small but in naturally green colour, and this visual symptom, besides Fe deficiency, is as a result of other nutrient deficiencies and diseases, accordingly it cannot be served as a clear-cut indicator of Fe deficiency in plants. But at more advanced stages of Fe deficiency, its visual symptoms become much clearer. At these stages, Fe chlorosis gradually appears and becomes evident. In our experiment, the colour of newly emerged leaves was natural, but their colour gradually turned yellow and finally chlorosis emerged (Fig. 1).

The result of this experiment revealed that Fe in pears' leaves was reduced by growing lime concentration. In other words, the content of chlorophylls was reduced as lime content in soil was increased. This evidence may suggest that, under Fe deficiency conditions, Fe chlorosis in leaves is associated with increasing pH of the soil and consequently of plants. It is worth mentioning that uptake of all nutrients in the soil is changed by increasing pH in soil. Therefore, we cannot claim confidently that leaf chlorosis is contributed to solely Fe deficiency under calcareous conditions.

The relationship between Fe content and chlorophylls content investigated in this research was in agreement with other researchers (Tagliavini, Rombola 2001). In this respect, due to an apparently insufficient Fe supply, the biosynthesis process of photosynthetic pigments will be impaired and enduring this status will finally lead to emerging Fe chlorosis symptoms. It seems that, at initial stage of Fe deficiency, producing new leaves with appropriate growth and sufficient Chl contents would be impaired due to accumulation of soluble bicarbonates ions in calcareous soils. This would remarkably influence physiological iron efficiency in leaves and roots. Finally, it would be followed by emerging yellowness and chlorosis symptoms in iron-deficient leaves. Reductions in leaf growth as well as emerging chlorosis were thus probably the symptoms of Fe-deficiency in plants grown in calcareous soils.

Therefore, the extant of leaf growth would not be depressed merely due to a reduction in photosynthesis products; however, it could occur because of a reduction in an available Fe to be used in shoots, internodes, and leaves. In general, findings of this research revealed that emerging severe chlorosis in plants depends on Fe deficiency (resulting from bicarbonates ions in calcareous soils) and the lack or reduction of photosynthesis products. Accordingly, our results are in agreement with those reported by Gonzalez et al. (2007).

In this research, it was demonstrated that presence of lime in such soils had significant negative effects on both Fe uptakes (total and available) in different rootstocks, and our findings are in consistent with those found by Zribi (2002).

Precipitation of a great deal of Fe in the leaves with Fe chlorosis indicates the lack of normal–Fe translocation into the targeted tissues by crossing cell plasmella membranes. Therefore, Fe accumulation in the apoplast of mesophyll and consequently depriving tissues being in a desperate need of Fe paves the way for occurring Fe chlorosis in leaves. It can be concluded that the factors such as high pH, high Fe (II) oxidation and low activities of Fe (III) redox enzymes are apparently contributed to the occurrence of Fe precipitation in sensitive leaves (Tagliavini, Rombola 2001).

The rate of *Fv*/*Fm* ratio is conversely related to the severity of stressful conditions. This relationship was elucidated in this research in a sense that when L concentration in soils was increased, the values of

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Fv/*Fm* in different R/S combinations was significantly diminished (Table 4). Environmental stresses such as lime and draught are indicators negatively affecting photosynthesis and related activities. Under such stressful conditions, a reduction in photosynthesis is accompanied with impaired in biochemical responses which were previously stated by Zhao et al. (2016). The ratio of *Fv*/*Fm* is generally related to capacity of electron translocation in photosystem (II). According to the results obtained by this research, an increase in lime concentration of soils (up to 10%), not only the values of *Fv*/*Fm* were diminished (Table 4), but also other variables including annual growth (AG), internode length (IL), and available Fe were negatively affected. Hence, it can be inferred that a reduction in Fv/Fm ratio represents stressful condition in plants.

A reduction in *Fv*/*Fm* ratio also indicates a decrease in photoreceptor, which can be interpreted as a harmful effect of lime on photosynthesis efficiency. In this regard, the results of this experiment are similar to those obtained by Zhao et al. (2016) where he found that exerting 25% full irrigation (FI) treatment in contrast to100% full irrigation significantly reduced the values of *Fm* and *Fv*/*Fm* ratio.

Selecting a precise Chl fluorescence variable (i.e. *Fv*/*Fm*) for evaluating the effects of different stresses (lime, salty, drought, and so on) on plants has been considered as an appropriate tool to ensure the accuracy of obtained data during stressful conditions. A reduction in value of Chl fluorescence indicates an increase in harmful effects of lime. Accordingly, this method has been considered as a simple, non-invasive, and fast method to monitor quality of mentioned rootstocks under calcareous soils. In general, measuring Chl florescence attributes along with other measurable parameters paves the way for identifying and introducing appropriate R/S combinations tolerant to different environmental stresses (lime, drought, etc.).

CONCLUSION

The results of this research revealed that the Rootstock/Scion (R/S) relationship plays a significant role in pear tolerance toward calcareous soils. Based on concentration of lime on soils, the response of pear's R/S combinations are different, in a sense that under low lime concentrations (10% control), many R/S combinations had better tolerance depending on the type of measured variable. However, at high lime concentration (20%), mainly the C5 combination (Pyrodwarf/Dargazi combination) showed significant tolerance to this condition.

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