RESEARCH ARTICLE

Leaf nutrient status of some grafted-pear rootstocks influenced by different soil types

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Abstract

Aim of study: Determining leaf nutrient status of some grafted-pear rootstocks grown under different calcareous soil types.

Area of study: The Horticultural Research Station of Kamalabad, Karaj, Iran.

Material and methods: Leaf mineral element concentrations were determined in 2015, 2016, 2017, and 2018. Leaf sampling was carried out about 90 days after full bloom. The N-content was estimated by the Kjeldahl method. Total concentration of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and boron (B) were also determined by the atomic absorption spectrophotometry, and phosphorus (P) and potassium (K) were analyzed using the flame photometry. The deviation from optimum percentage (DOP) and its correlation with the ΣDOP of macro- and micro-nutrients were used to determine the nutritional status of the studied plants.

Main results: In clay loamy soil pyrodwarf rootstock, which was grafted with 'William Duchess' scion, presented a more suitable balanced nutritional index than other rootstocks/scion combinations. The OHF69 rootstock grafted with 'Daregazi' offered better balanced nutritional values in fairy lime silt-loamy soil, whereas the pyrodwarf rootstock grafted with 'Louise Bonne' scion in less lime silt-loamy soil was found to have higher values than other studied rootstocks/scion combinations in terms of nutrient concentration.

Research highlights: The study determined effective solutions to the field problems of calcium carbonate equivalent which distinctly affect the soil properties related to plant growth. It also revealed the most suitable pear rootstock/scion combinations in different calcareous soil types for orchard establishment in arid and semi-arid regions.

Additional key words: calcium carbonate equivalent; deviation from optimum percentage; ΣDOP index

Abbreviations used: DAFB (days after full bloom); DOP (deviation from optimum percentage index); EC (electrical conductivity); OC (organic matter); SP (saturation percentage); TNV (total neutralizing value).

Introduction

Pear rootstocks may come from several species of *Pyrus* or a different genus. Depending on environmental conditions, the response of each pear rootstock and scion combinations is different (Ikinci *et al.*, 2014). While semi-dwarf growing Pyrodwarf and OHF69 rootstocks are generally well-adapted to the high lime content soils, their responses vary drastically according to pear scion/various rootstocks combinations (Bosa *et al.*, 2014). Calcareous soils are widely spread in arid and semi-arid regions, which have been estimated to comprise over one-third of the world's land surface area (Taalab *et al.*, 2019). Reported by the Iranian Ministry of Agriculture (2013), the calcareous soils constitute up to 60% of the total area of Iran. The presence of calcium carbonate ($CaCO_3$) in the parent material and the accumulation of lime are crucial factors that identify calcareous soils. This is recognized conveniently by the effervescence (fizzing) when these soils are treated with diluted acid. The pH of these soils is usually above 7.0 and may be as high as 8.5 and when contain sodium carbonate, their pH may exceed 9.0. In some soils, $CaCO_3$ can also concentrate into very hard layers, termed as

'caliche', which is impermeable to water and plant roots (Taalab *et al.*, 2019).

Iran is located in the arid and semi-arid region of the world, like some other countries such as Pakistan, Indus Basin, Iraq, Jordan, and Lebanon in the Near East and also Portugal, Spain, Italy and Greece in southern Europe. A large proportion of the cultivated lands in this country consists of calcareous soils (FAO, 1977). Such soils have high levels of calcium carbonate equivalent and pH that all together contribute to growth reduction, lower yield, nutrient deficiencies, and leaf-chlorosis (Gharaie, 2009; Dilmaghani *et al.*, 2012). According to the recent statistics, an area of 2.632 ha in Iran is under pear culture with an average annual total production of up to 80.576 tones (FAO, 2017). This average of production, however, is still low since the majority of planted pear trees are located in provinces with high lime soils such as Tehran, Alborz, and Isfahan. These soils are calcareous and contain high pH and calcium carbonate equivalent. In addition, the amount of lime soils in these regions is another issue of concern. Although the nutritional status of pear trees is adversely affected by 14% of soil lime, amount of lime in these regions varies in a range of 10 to 14 % (Ikinci *et al.*, 2014). From the point of leaf nutrient status, the Deviation from the Optimum Percentage (DOP) method is an interpretation method to compare nutrient concentrations with the references using a percentage expression (Montañés *et al.*, 1993; Lucena, 1997). This method quantifies the difference between a single nutrient concentration and its reference value, offering the advantage of ranking the requirements or limitations from the most negative to the highest positive nutrient index (Montañés *et al.*, 1993). Furthermore, the sum of the absolute value of the different DOP indexes ($\Sigma|DOP_i|$) is a general index which represents the complete nutritional balance of the plant and the severity of an anomalous situation. In this study, the behavior of OHF69, pyrodwarf, and one pear seedling rootstock grafted with 'Daregazi', 'Louise Bonne,' and 'William Duchess' scions is determined in three different soil types (less lime silt-loamy, with 10.5% calcium carbonate equivalent; fairy lime silt-loamy, with 12.9% calcium carbonate equivalent; and clay loamy, with 14.6% calcium carbonate equivalent) in the Alborz province over four years (2015, 2016, 2017, and 2018) using leaf nutritional status and the DOP and ΣDOP indexes about 90 days following full blossom.

Material and methods

Plant materials

The 'Daregazi', 'Louise Bonne' and 'William Duchess' scions were chip-budded at 10 cm height of one-year-old of three rootstocks (OHF69, pyrodwarf and a seedling of *Pyrus communis*, obtained from local wild pear genotype) in 2013. Then, they were grown under nursery of Cold and Temperate Fruits Research Center. In winter of 2014, the plant materials were transferred to the experimental orchard located at the Kamalabad Research Station, Karaj, Iran and then, were grown under three different soil types (Table 1) over four years (2015 – 2018).

Geographical location and the weather conditions

The Horticulture Research Station of Kamalabad is located in Karaj, Alborz, Iran (50° 52' East latitude, 35° 52 'north latitude) with 1320 m height above mean sea level, average annual temperature of 14°C, and the average rainfall of 254.5 mm per year.

Soil treatments

The soil of Horticultural Research Station of Kamalabad consists of four soil series: soil series 1=Xeric Torriorthents, less loamy, mixed (calcareous) thermic; soil series 2=Xeric haplocalcids, fairy loamy, mixed, thermic; soil series 3=Xerifluventic haplocalcids, clay loamy, mixed, thermic; soil series 4=Xeric haplocampids, fine, mixed,

Table 1. Physico-chemical analyses and mineral substance content of the soil of an experimental orchard

Soil type[1]	Ava. K-soil	Ava. P-soil	Soil pH	EC[2] (dS/m)	Soil particle (mm)			SP[3]	OC[4]	N-Soil	TNV[5]
	(mg kg⁻¹)				Sand 2-0.05	Silt 0.05-0.002	Clay <0.002	%			
A	740	5	8	0.8	17	55	28	39	0.60	0.07	10.5
B	580	17.4	8.1	0.69	20	48	32	38	0.30	0.12	12.9
C	570	10	7.9	1.9	44	30	28	38	0.18	0.05	14.6

[1] Soil types: A, less lime silt-loamy (with 10.5% calcium carbonate equivalent), B, fairy lime silt-loamy (with 12.9% calcium carbonate equivalent) and C, clay loamy (with 14.6% calcium carbonate equivalent). [2]EC= Electrical conductivity. [3]SP= Saturation percentage. [4]OC= Organic matter. [5]TNV= Total neutralizing value (soil lime)

thermic. These soil series contain different soil lime levels, according to detailed excavation reports by Fallahi (1998). The soil physicochemical analysis (Walky & Black, 1934; Drouneou, 1942; Isaac & Kerber, 1971; Olsen & Sommers, 1982) was analyzed prior to the orchard establishment. Surface soil (0-30cm) variables were tested to clarify the percentages of Total Neutralizing Value (TNV), total N, pH, available K and P (me L^{-1}) and physical parameters such as the percents of silt, sand, clay, and saturation percentage (SP) as well as organic matter content (Table 1).

Experimental design

The field experiment was conducted on a split-factorial arranged based on the randomized complete blocks design (RCBD) with three replications. Measurements were performed at 90 DAFB over four growing seasons including 2015, 2016, 2017, and 2018. Three field-collected soil types were considered as the main plots and three grafted-pear rootstocks were assigned in the sub-plots. Each soil trial consisted of three blocks with three rows. Each row contained 27 grafted pear rootstocks. Data were collected from the nine central trees in each block, using the remaining trees as guards. The plants grafted with the OHF69, pyrodwarf rootstocks were spaced at 3 m × 1 m intervals, the plants grafted with the seedling were spaced at 3m × 3m, headed at 80 cm and trained according to the modified leader system.

Cultural treatments

A computerized drip irrigation system was applied twice per week from May to October using a class-A pan according to the regional recommendations. Each treatment (grafted-pear rootstocks in each studied soil series) received the same amount of water in each growing season. All trees were also fertilized with essential minerals using the same fertigation method. Weed, disease, and pest controlling were carried out using the protocols commonly used for commercial production.

Analysis of leaf macro- and micro-nutrient composition

The concentrations of leaf mineral elements were determined at 90 DAFB in 2015, 2016, 2017, and 2018. The samples (10 leaves per plant) were collected from young expanded leaves of all grafted pear rootstocks. The nitrogen content was estimated by the Kjeldahl method. The Ca, Mg, Fe, Zn, and B were determined using an atomic absorption spectrophotometry {Association of Official

Analytical Chemists (AOAC) 2016}. Phosphorous (P) was analyzed by the molybdovanadat method using a Jenway 6305UV–VIS. Potassium (K) was also analyzed by the flame photometry using a Jenway PFP7 flame photometer (Jenway, Essex, UK). The DOP indexes of macro- and micro-nutrients were used to determine nutritional status of fruit trees: normal (DOP=0), deficiency (DOP < 0) and excess (DOP > 0) (Montañés et al., 1993). The DOP index was calculated from the leaf analysis in July of each year by the following mathematical equation:

$$DOP = \frac{C \cdot 100}{C_{ref}} - 100$$

where C is the measured nutrient content in the sample, and C_{ref} is the major nutrient content considered as optimum, which both values are given on a dry matter basis. The C_{ref} was taken from optimum values, proposed by Bergmann (1992) for nutrients (Table 2). The ΣDOP for four studied years was obtained by adding the values of the DOP index irrespective of the sign. The larger ΣDOP was the intensity of imbalances among nutrients.

The SAS and SPSS statistical software were used to calculate the surveyed data. The relationships between ΣDOP and DOP indexes of each evaluated soil type over four studied years were also evaluated by the Spearman correlations.

Results and discussion

Responses of leaf macro- and micro-nutrient composition of some grafted-pear rootstocks grown under different calcareous soil types over four years (2015, 2016, 2017, and 2018)

Calcareous soils contain high levels of CaCO$_3$ which affect soil properties related to plant growth such as soil water content and the availability of plant nutrients

Table 2. Critical levels of nutrients in the pear leaf used as reference (Bergmann, 1991) for calculating the DOP statistical analysis.

	Critical level
Macro-nutrients (%)	
N	2.2
P	0.15
K	1.2
Mg	0.2
Ca	1.2
Micro-nutrient (mg/kg)	
B	20
Fe	250
Zn	20

(Elgabaly, 1973). They are common in the world's arid areas (FAO, 2016) occupying more than 30% of the total earth's surface, the majority of which are located in the Near East and the southern Europe (FAO, 1977). Their $CaCO_3$ content also varies from just detectable up to 95% (Marschner, 1995). Two-thirds of Iran is arid with Calcic Yermosols and strongly calcareous Haplic Yermosols and also Calcic Xerosols, thus affecting more than 60% of the soil (Dewan & Famouri, 1964). Such soils are identified by the presence of the mineral $CaCO_3$ or lime in the parent materials and the accumulation of lime. The pH of these soils is usually above 7.0 and can be as high as 8.5. Several land evaluation studies for different crops in Iran demonstrated that soil aridity, salinity, and high carbonate content in soils are among the most serious limiting factors in arid and semi-arid lands (Moghimi, 2002; Garkaninegadmasizi et al., 2009). Variation in leaf nutrient status of pear rootstock/scion combination can be ascribed to the different types of soils applied (Lewko et al., 2004; Elkins et al., 2011, 2012; Bell et al., 2012; Elkins, 2012; Milošević1 & Milošević, 2016). Reported by Jacobs & Cook (2003) and Bosa et al. (2014), physiological aspects, growth parameters, and some leaf nutrition content of pear trees are negatively regulated by high pH and lime-rich clay loamy, although the response varies according to pear scion/various rootstocks combinations. In the present study, the effects of three soil types collected from the different soil series of Horticultural Research Station of Kamalabad, Karaj, Iran at the depth of 0-30 cm exhibited significantly different responses of leaf nutrient status in the scion/rootstocks combinations. Moreover, statistical analysis indicated that the factors including year, soil type, and grafted-pear rootstock, alone and in combination, exhibited significant effects on leaf mineral content (Table 3). These results have also been confirmed by Stassen & North (2005), Erdal et al. (2008) and Marschner (2012), who proved many factors affecting the nutritional status of a plant, which can be classified to three main groups such as soil, environment, and plant factors.

Leaf mineral nutrients and DOP index

To determine the best pear scion/ rootstocks combinations for different soil types, the DOP and ΣDOP indexes were measured from leaf mineral elements at 90 DAFB. The following observations by the aforementioned indexes were recorded during the 4 years course of experimentation.

Deviation from optimum percentage (DOP index) and ΣDOP index

Based on the DOP index, all studied leaf nutrient contents were either lower or higher than optimum level. The grafted pear rootstocks grown under different soil types in all studied years, except for DOP_P level {in pear seedling and OHF69 rootstocks in grafting with 'Louise Bonne' in soil type C (lime rich clay loamy), and in pyrodwarf rootstock grafted with 'Louise Bonne' in soil type B (fairy lime silt-loamy)}, the DOP_B level {in OHF69 rootstocks in grafted with 'Louise Bonne', pear seedling rootstocks grafted with 'William Duchess' in soil type C (lime rich clay loamy) in 2016}, the DOP_{Mg} level {in pyrodwarf and pear seedling rootstocks grafted with 'William Duchess' in soil type B (fairy lime silt-loamy)}, the DOP_{Ca} level {in pear seedling rootstocks grafted with 'William Duchess' in soil type A (less lime silt-loamy), the DOP_B level {'William Duchess' in soil type B (fairy lime silt-loamy), and finally, the DOP_{Fe} level {OHF69 rootstocks grafted with 'Louise Bonne' in soil type C (lime rich clay loamy)} in 2018 with a DOP value close to the normal level (Table S1 [suppl.]).

This situation can be explained by the verity that scions may differ in nutrient content due to differential nutrient absorption and/or translocation (Milošević et al., 2013). Table S1 [suppl.] has also indicated significant differences among soil types within the same grafted-pear rootstocks for nutritional balance or ΣDOP index. The high variability of ΣDOP index shown by the leaf nutrient content has already been reported by Zarrouk et al. (2005), Milošević et al. (2014), and Milosevic & Milosevic (2011b,c). In addition, Milošević & Milosevic (2011a, 2015, and 2016) clarified different behavior of pear, apple, and plume cultivars on various rootstocks relates to leaf macro- and micronutrients content.

Spearman correlations analyses

Mean values of all studied grafted pear rootstocks over four years (2015, 2016, 2017, and 2018) were used for Spearman correlation analysis between ΣDOP and DOP indexes (Table 4), and each evaluated nutrient of different soil types was classified as follows:

—Highly positive ($0.45 \leq r < 0.90$) with the DOP_P, DOP_{Ca}, and DOP_{Mg}, the index for soil type A (less lime silt-loamy soil with 10% calcium carbonate equivalent).
—Highly negative ($-0.45 \leq r < 0.90$) with the DOP_{Zn} index for soil type A (less lime silt-loamy soil with 10% calcium carbonate equivalent).
—Highly positive ($0.45 \leq r < 0.90$) with the DOP_P and DOP_{Ca} the index for soil type B (in fairy lime silt-loamy soil with 12% calcium carbonate equivalent).
—Moderately positive ($0.337 \leq r < 0.45$) with the DOP_{Mg} index.
—Moderately negative ($-0.337 \leq r < 0.45$) with DOP_K for soil type B (in fairy lime silt-loamy soil with 12% calcium carbonate equivalent).

Table 3. Combined analysis of variance for the leaf nutrition concentration of studied grafted-pear rootstocks grown under different soil types in four studied years (2015, 2016, 2017, and 2018)

Source	DF	Mean square							
		N	P	K	Ca	Mg	B	Fe	Zn
		(%)					(ppm)		
Soil type	2	3.97**	0.37**	1.38**	0.05**	0.16**	28.83**	1184.72**	46.53**
Year	3	43.82**	10.89**	9.81**	2.81**	1.58**	12847.82**	485399.95**	2951.12**
Soil type*Year	6	0.57**	0.16**	0.62**	0.06**	0.10**	14.163**	24076.37**	74.05**
Block*Soil type*Year	24	0.01**	0.02**	0.01**	0.3**	0.06**	0.16**	91.12**	0.72**
A (rootstock)	2	2.88**	0.19**	0.74**	1.39**	0.03**	354.55**	21949.01**	31.24**
Soil type*A	4	0.46**	0.16**	3.28**	0.52**	0.06**	21.97**	13701.39**	161.72**
Year*A	6	2.09**	0.70**	2.24**	0.26**	0.11**	101.27**	99955.68**	248.20**
Place*Year*A	12	0.67**	0.17**	1.60**	0.03**	0.17**	23.92**	6582.00**	103.41**
A*Block (Block*year)	48	0.01^{ns}	0.003^{ns}	0.02^{ns}	0.01^{ns}	0.04^{ns}	0.17^{ns}	91.57ns	0.75^{ns}
B	2	1.48**	0.81**	4.42**	1.21**	0.87**	346.08**	20877.71**	652.98**
A*B	4	4.40**	0.28**	5.67**	0.91**	0.74**	378.06**	24257.18**	232.55**
Year*B	6	4.61**	0.19**	2.392**	0.07**	0.41**	226.49**	28523.42**	134.94**
Place*B	4	0.33**	0.47**	1.42**	1.51**	0.42**	94.36**	5978.79**	288.37**
Place*Year*B	12	0.23**	0.16**	0.52**	0.1**	0.06**	19.66**	3190.68**	55.62**
Year*A*B	12	2.36**	0.25**	3.71**	0.43**	0.29**	106.62**	104494.31**	179.88**
Place*A*B	8	1.09**	0.18**	1.39**	0.14**	0.31**	25.63**	11082.98**	138.94**
Place*Year*A*B	24	0.44**	0.16**	0.42**	0.15**	0.09**	21.21**	10096.90**	31.00**
CV (%)		3.18	14.12	0.83	10.99	36.29	2.04	4.09	3.38

p*≤0.05; p**≤0.001, respectively; ns, no significant (F-probability).

—Highly positive ($0.45 \leq r < 0.90$) with the DOP_P and DOP_{Mg}, the index for soil type C (clay loamy soil with 14% calcium carbonate equivalent).
—Moderately positive ($0.337 \leq r < 0.45$) with the DOP_B and DOP_N index for soil type C (clay loamy soil with 14% calcium carbonate equivalent).

Working on conilon coffee crop, Fonseca *et al.* (2018) obtained positively high and significant Spearman correlations ($p \leq 0.05$) between ΣDOP and the DOP indexes of Mn; moderately positive with B; moderately negative for DOP index of P, K, S, and Zn. Our results differed for every single studied soil type. According to the results from to the Spearman correlation, the nutritional imbalance occurred due to the excess of P and Ca, Mg, and Zn deficiency in the leaves of the evaluated pear rootstock/ scion combinations in soil type A. The pyrodwarf rootstock grafted with 'Louise Bonne' scion, however, was more efficient in soil type A (less lime silt-loamy soil (with 10% calcium carbonate equivalent). In the B-type soil (fairy lime silt-loamy with 12% calcium carbonate equivalent), nutritional imbalance occurred due to the excess of P and Ca, Mg and the K deficiency in

the leaves of the evaluated pear rootstock/ scion combinations, whereas the OHF69 rootstock grafted with 'Daregazi' scion, followed by OHF69 rootstock grafted with 'Daregazi' were more efficient in soil type B. The best balanced nutritional values in the C-type soil {clay loamy (with 14% calcium carbonate equivalent)} were found in the pyrodwarf rootstock grafted with 'William Duchess' scion, followed by OHF69 rootstock grafted with 'Daregazi'. Veloso *et al.* (1995) noted the difficulty of studying the effects of different nutrient toxicity alone, mostly due to interactions with other elements. These interactions can be responsible for the diversity of symptoms and different degrees of growth reduction in different species and cultivars (Foy *et al.*, 1978). The Spearman correlation analyses proved the strong relation between the element levels in leaves. This information is very important to find the most suitable pear rootstock/ scion in combinations with different calcareous soils for orchard establishment in the arid and semi-arid regions across the world, like some countries in the Near East (Pakistan, Indus Basin, Iran, Iraq, Jordan, and Lebanon), and also for countries of southern Europe, such as Portugal, Spain, Italy, and Greece.

Table 4. Spearman correlation analysis between ΣDOP and DOP indexes of each evaluated soil type. Values are the mean of all studied grafted-pear rootstocks over five years (2015, 2016, 2017, and 2018).

	Soil	DOP								[1] ΣDOP
		N	p	k	Ca	Mg	B	Fe	Zn	
ΣDOP	Soil A	0.24	**0.717****	-0.159	**0.545****	**0.669****	0.185	0.185	**-0.435****	1
	Soil B	0.161	**0.917****	-0.337*	**0.478****	**0.414***	0.249	0.179	-0.21	1
	Soil C	**0.410***	**0.806****	0.247	0.135	**0.504****	**0.398***	0.072	0.135	1

[1] ΣDOP: sum of the absolute value of the different DOP indexes. *,**: $p \leq 0.05$, $p \leq 0.001$, respectively (F-probabilities)

This paper presents creative data concerning the content of eight micro- and macro-elements in leaves of 'Daregazi', 'Louise Bonne' and 'William Duchess' scions grafted with OHF69, pyrodwarf, or a seedling of *Pyrus communis* which were grown in three different soil types (less lime silt-loamy, fairly lime silt-loamy and lime-rich clay loamy) over five years (2015, 2016, 2017, and 2018). Among all studied rootstocks, the pyrodwarf rootstock showed a more adequate level of mineral nutrients in the loamy soil. However, among all studied scions, 'Louise Bonne' scion showed a more adequate level of mineral nutrients in the loamy soil. Pyrodwarf rootstock grafted with 'William Duchess' scion exhibited a relatively more suitable balanced nutritional index than other rootstocks/ scion combinations in clay loamy soil with 14% calcium carbonate equivalent. Our results also revealed that the seedling rootstock grafted with 'Louise Bonne' resulted in superior balanced nutritional values in the fairy lime silt-loamy soil with 12% calcium carbonate equivalent. The pyrodwarf rootstock grafted with 'Louise Bonne' scion was more efficient in less lime silt-loamy soil with 10% calcium carbonate equivalent. This rootstock when grafted with 'Louise Bonne' scion showed the lowest ΣDOP (290.33) and the greatest intensity of balance among nutrients than other rootstocks/scion combinations in less lime silt-loamy soil. In comparison to other rootstocks/ scion combinations in fairy lime slit-loamy soil, the OHF69 rootstock in grafting with 'Daregazi' showed the highest ΣDOP (398.44) and the lowest intensity of balance among nutrients.

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