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Performance of Miyagawa Satsuma Mandarin Raised on Swingle citrumelo in Calcareous Soils

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ABSTRACT

In a two-year experiment, the performance of Miyagawa satsuma mandarin was evaluated on Swingle citrumelo rootstock on soils with different amounts of lime. In a field experiment, plant growth trend, yield, and quality were studied in two regions (plain and piedmont) for 10 years. In the plain experiment, the highest shoot dry weight was obtained from soils with 14% total lime. In soils with more total lime, however, shoot dry weight decreased sharply. The highest chlorosis rate was obtained in soils with more than 30% lime. The highest total Fe in the roots, together with the least signs of leaf chlorosis, were detected in soils with less than 9% lime. The average Fe concentration in the roots was about 7.5 times more than that in the leaves, indicating accumulation and deposition of Fe in the roots. The amount of available Mn was excessi-ve in most soils, but leaf Mn in most cases was less than adequate. The Mn concentration in the roots was 3.2 times more than that in the leaves. Tree volume in the field was always higher than that in the piedmont. Trees in the piedmont had alternate bearing cycles. The yield increased from 23 kg per tree at the beginning of the reprodu ctive period to 80 kg in the final year of the experiment. The best harve st time in both regions was early October. Generally, use of this rootstock in soils with less than 9-14% total lime is recommended.

Introduction

The citrus cultivation area in the north of Iran is about 110000 hectares with over 2.5 million tons of annual production. Evaluations of soil in the north of Iran have shown that the main physiography is piedmont alluvial plains (loess sediments) downstream of the heights along with the southern parts, river alluvial plains (heavy to medium texture) that continue to the middle of the plain, basin of river interfluves with medium to light texture and shallow river alluvial with a light texture that is located on coastal sediments with very heavy textures, coastal low lands with a light texture that are located along the Caspian Sea and form a narrow margin (Asadi and Akhlaghi, 2020).

Creating a sustainable production system is the main challenge of fruit science nowadays. Decline in soil fertility due to nutrient mining is the major constraint, limiting the productivity of fruit crops (Srivastava and Hota, 2020). One of the most important chemical properties of soil that impairs the availability of Fe for plants is calcium carbonate, which is present in more than 30% of the world's land (Loeppert et al., 1994). In the north of Iran, the amount of soil calcium carbonate in the orchards increases gradually from the middle to the east (Fig. 1).

Soil texture, calcium carbonate, and organic matter are the most important soil physical properties, playing a role in aeration and Fe absorption. However, the results of soil network studies on the availability of Fe (Fig. 2) showed that the amount of plant-available Fe in the soil of

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most orchards in the north of Iran is more than sufficient (Asadi and Akhlaghi, 2015).

Fig. 1. Distribution of calcium carbonate in soils of Mazandaran province in the north of Iran. Yellow, 0-5%; pale blue, 5-10%; brown, 10-15%; green, 15-20%; pink, 20-30%; bold blue, 30-50%; gray more than 50% of the equivalent calcium carbonate (Asadi and Akhlaghi, 2020).

Fig. 2. Distribution of plant-available Fe (extracted by DTPA method) in soils of Mazandaran province in the north of Iran. Pale green, 0-0.5 mg kg^{-1}; Bold green, 2.5-5 mg kg^{-1}; Purple, 5-7.5 mg kg^{-1}; Pink, 7.5-10 mg kg^{-1}; Pale orange, 10-20 mg kg^{-1}; Orange, 20-40 mg kg^{-1} and bold orange, more than 40 mg kg^{-1}, plant-available Fe (Asadi and Akhlaghi, 2015).

Currently, Swingle citrumelo and different cultivars of citrange serve as rootstocks in the north of Iran. Most citrus cultivars, and blood oranges in particular, with these rootstocks, do not have good yield and quality in the soils with high calcium carbonate (Asadi and Akhlaghi, 2020). Swingle citrumelo tolerates cold weather and moderate drought, but shows chlorosis in calcareous soils. In the soils with more than 25-30% clay, root growth of Swingle rootstock is limited. Swingle citrumelo is unsuitable for heavy clay soils with excessive irrigation (Castle and Stover, 2001). Field observations showed that trees on Swingle citrumelo rootstock initially grew well in calcareous soils with heavy texture but began to decline after five to seven years, with substantial losses in vigor and productivity (Stover et al., 2002). The use of Swingle citrumelo as a rootstock in calcareous soils with heavy texture is limited due to its inability to adequately absorb microelements such as Fe (Pestana et al., 2005; Castle and Nunnallee, 2009). In Turkey, calcareous soils caused severe Fe chlorosis in Swingle citrumelo (Meral et al., 2015). Prediction of Fe chlorosis in fruit trees during orchard planting is very important, and any mistake makes it impossible to gain desirability in yield (Loeppert et al., 1994).

Considering the large changes in lime in the soils of the north of Iran along with the expanding use of Swingle citrumelo as the main rootstock in citrus orchards of north of Iran, evaluations on the response of Swingle citrumelo rootstock to the different amounts of lime is essential. Moreover, one of the common problems is the interest of orchardists in early fruit harvest for early-maturing mandarin before reaching the standard level of fruit harvest index. Rootstocks can affect both harvest time and fruit quantity and quality (Castle and Stover, 2001). Therefore, determining the appropriate time to start harvesting and other field performances for Miyagawa satsuma mandarin on Swingle citrumelo rootstock were the objectives of this study.

Materials and Methods

Pot experiment

According to soil maps and several studies in the north of Iran (Asadi, 2019), seven soil samples were selected from a geographically large part and major citrus cultivation areas (Table 1). The range of equivalent calcium carbonate of soils varied in the range of 2-45%, with active calcium carbonate (0-16%) (Bashour and Sayegh, 2007), clay (13-41%), silt (18-37%), sand (34-58%) (Gee and Bauder, 1986), and organic carbon (0.65-1.8%) (Nelson and Sommers, 1982). Then, 28 uniform seedlings of Miyagawa satsuma mandarin (*Citrus unshiu*) on two-year-old Swingle citrumelo rootstock (with 50 cm height and one cm diameter) were planted in pots containing 30 kg soil. The pots were kept in a plastic greenhouse. The experiment was carried out in a randomized complete block design with seven soils containing different ranges of lime (2-45%) and four replications for two years. During the growth period, fertigation was regularly done every other week with potassium nitrate (1.4 mmol l^{-1}), potassium sulfate (0.6 mmol l^{-1}), magnesium sulfate (1 mmol l^{-1}), mono ammonium phosphate (0.6 mmol l^{-1}), ammonium sulfate (3 mmol l^{-1}), and ammonium molybdate (1 μmol l^{-1}). Irrigation was done with the random weighing of pots (Boman et al., 2008). Leaf samples were prepared in August from the middle part of the growing shoots (Asadi and Akhlaghi, 2020).

To estimate the severity of chlorosis, a rating scale (Byrne et al., 1995) was used in the second year (Table 2). Plant responses included vegetative growth trend, dry weight, leaf chlorosis index, the relationship between active lime in soil and active Fe in leaves, total Fe concentration, active Fe, Mn, Zn, and Cu in leaves and roots, as well as the relationships among them.

Table 1. Some physical and chemical properties of the soils

	Soils						
	1	2	3	4	5	6	7
Clay (%)	23	29	19	41	13	37	23
Silt (%)	30	26	35	18	29	29	37
Sand (%)	47	45	46	41	58	34	40
Texture	loam	sandy clay loam	loam	clay	sandy loam	clay loam	loam
Lime (%)	2	9	14	30	40	25	45
Active lime (%)	0	3	5	14	7	10	16
Organic matter (%)	1.17	0.95	1.80	1.60	0.65	1.52	1.10
pH	6.8	7.45	7.86	7.60	7.77	7.78	7.76
P (mg kg^{-1})	26	22	15	17	11.20	18.30	9.87
K (mg kg^{-1})	404	380	360	460	221	325	265
Fe (mg kg^{-1})	7.20	6.40	8.80	8.90	4.40	8.22	6.80
Mn (mg kg^{-1})	3.10	4.20	3.96	5.40	3.20	7.71	3.40
Zn (mg kg^{-1})	2.40	2.50	0.7	0.6	0.91	1.60	1.50

Table 2. Guidance for leaf chlorosis rating of seedling their fully expanded new leaves.

Leaf chlorosis rate	Symptoms
1	Healthy green leaves
2	Yellowish-green inter-veinal areas, green veins
3	Greenish-yellow inter-veinal areas, green veins
4	Yellow-inter-veinal areas, green veins
5	Yellow-white inter-veinal areas, pale green veins, some defoliation

Field experiment

The effects of Swingle citrumelo rootstock on vegetative and reproductive characteristics of Miyagawa satsuma mandarin were evaluated in a separate experiment. The experiment was performed in a randomized complete block design with four replications, with four trees in each replication at two locations (plain and piedmont) during 2009-2018 (Table 3).

Table 3. Some characteristics and long term meteorological features of locations of the experiment

	Location 1 (plain)	Location 2 (piedmont)
Latitude (° N)	36° 27′	36° 31′
Longitude (° E)	52° 53′	53° 31′
Altitude (m)	15	118
Soil texture	loamy	clay-loam
Soil total lime (%)	11	3
Soil pH	7.8	6.2
Annual rainfall (mm)	719	745
Maximum temperature (°C)	23.3	24.4
Minimum temperature (°C)	12.5	11.1
Absolute maximum temperature (°C)	40.6	42.6
Absolute minimum temperature (°C)	-6	-8.4
Relative humidity (%)	79	70

In the spring of 2009, Miyagawa mandarin buds were grafted on Swingle citrumelo rootstock, and in the spring of 2010, young trees were planted in the main fields with a distance of 5×4 m. At the same time, a drip irrigation system was installed in both locations. Maintenance of trees during the experimental period was performed almost identically in both locations. To stimulate the vegetative growth of the young trees, all the flowers were mechanically removed during 2011-2013. All young trees were trained in 2011-2013 and, after the beginning of the reproductive phase, annual winter pruning was performed on all trees until the end of the experiment (Akhlaghi, 2019). Each year (2011-2018), some vegetative characteristics, i.e. tree height, canopy diameter (average length and width of canopy), and trunk perimeter at the top and bottom of graft union were measured (Tazima et al., 2013). The yield of all 32 trees was recorded from the beginning of the reproductive phase to the end of the experiment at harvest time (mid-October, 2014-2018). In mid-September and early October of 2014-2016, 10 fruits from each tree were harvested randomly and some fruit characteristics were measured. Then, measurements were aimed at harvest index, juice, and peel percentage (Tazima et al., 2013). The volume of the canopy (m^3), yield efficiency (kg m^{-3}), and cumulative yield (2014-2018) were calculated (Stenzel and Neves, 2004). Alternate bearing index (2014-2018) was measured (Monselise and Goldschmidt, 1982). The theoretical area of trees, the theoretical number of trees, and the theoretical yield (per hectare) were calculated according to a method by Tazima et al. (2013).

Results

Pot experiment results

Dry weight

The results showed that the highest and lowest shoot dry weight of Miyagawa satsuma mandarin on Swingle citrumelo rootstock were obtained from soils with 14 and 45% lime, respectively. Also, the highest and lowest root dry weight was measured in soils with 14 and 40% lime, respectively (Fig. 3).

Leaf chlorosis index

The chlorosis rate of plants in different soils is shown in Figure 4. The highest chlorosis was obtained in soils with 30 and 45% lime and the lowest was obtained in soils with 2 and 9% lime. Soils with 30 and 45% lime had the highest active lime and caused the most severe chlorosis.

Concentrations of microelements in roots and leaves

The results of total and active Fe, Mn, Zn, and Cu in the leaves and roots of Miyagawa satsuma mandarin on Swingle citrumelo rootstock (Tables 4 and 5) showed that total Fe in the roots was much higher than in the leaves (946 and 127 mg kg^{-1}, respectively). Also, the transfer coefficient of microelements (Fig. 5) showed that total Fe had the lowest transfer coefficient, whereas active Fe had the highest transfer coefficient from roots to shoots. Cu, Mn, and Zn followed after total Fe, respectively. Total Fe in the roots in different soils was 7.45 times higher than in the leaves, indicating that most of the absorbed Fe accumulated in the roots. This is consistent with the results of other researchers who reported that in calcareous soils most of the absorbed Fe is deposited and stored in the apoplasts of root cells (Mengel, 1995; Kosegarten et al., 1999).

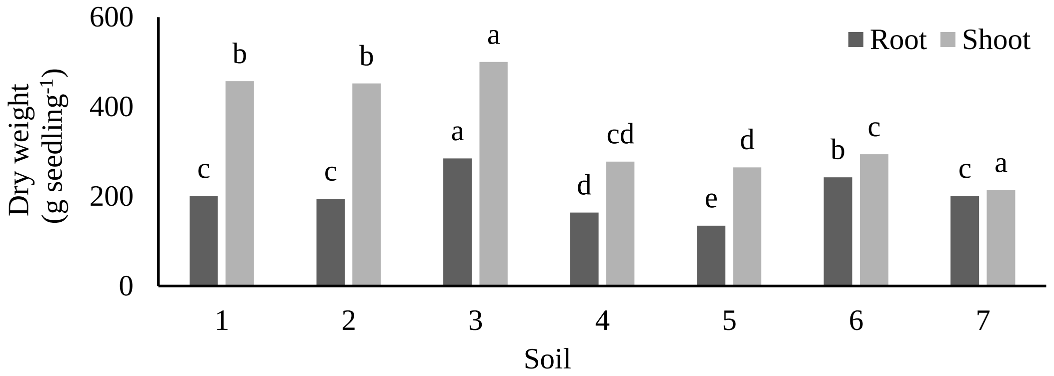

Fig. 3. Dry weight of shoots and roots in Miyagawa satsuma mandarin on Swingle citrumelo in different soils (Mean values with the same letters are not significantly different at 5% probability level).

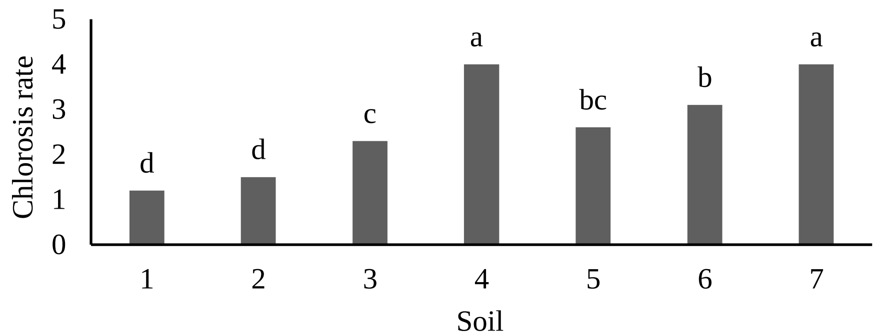

Fig. 4. Chlorosis rate in Miyagawa satsuma mandarin on Swingle citrumelo rootstock in different soils. (Mean values with the same letters are not significantly different at 5% probability level).

Active Fe in the leaves and roots varied in different soils. Soils with 40% lime had the lowest active Fe in the leaves, but the highest content in the roots (18 and 61 mg kg^{-1}, respectively). In general, active Fe in the roots and leaves in different soils were 38.85 and 40.8 mg kg^{-1}, respectively, and there was no significant difference between the active Fe in the roots and leaves. The soils with 2, 9, and 45% lime had the highest Mn in the roots (47, 48, and 52 mg kg^{-1}, respectively). However, leaf Mn content was low in all soils, (5.9-34.9 mg kg^{-1}) which is much less than the optimal concentration of Mn in the leaves of Satsuma mandarins (Asadi, 2019). The Mn in different soils was 42.71 mg kg^{-1} in the roots and 13.34 mg kg^{-1} in the leaves, which shows that Mn in the roots was 3.2 times higher than in the leaves. The results of Zn concentration in the roots and leaves showed that the highest Zn contents in the leaves and roots were obtained in soil 5 with 40% lime, which was 31 and 161 mg kg^{-1}, respectively. Zn in the roots and leaves in different soils was 103 and 24.57 mg kg^{-1}, respectively, which shows that Zn content in the roots was 4.19 times higher than in the leaves (Asadi, 2021).

Field experiment results

During the field experiment (2011-2018), all vegetative traits, i.e. tree height, canopy diameter, trunk perimeter in the trees of the plain (location 1) were always higher than those in the trees of the piedmont (location 2). So, the volume of trees in location 1 was always higher than that in location 2 (Fig. 6). In general, the plain had more soil depth than the piedmont and this caused a better expansion of the tree canopy. Edriss et al. (2019) found a strong correlation between canopy volume and climatic conditions. The affinity of scion and rootstock during 8 year-changes was bell-shaped (Fig. 7) and not significantly different in the two locations. Fadel et al. (2019) reported that the rootstock/scion trunk diameter ratio in Swingle citrumelo and Pera sweet orange did not influence fruit yield and quality.

Table 4. The concentration of microelements in Miyagawa satsuma mandarin on Swingle citrumelo rootstock in different soils. (Mean values for each column with the same letters are not significantly different at 5% probability).

Soils	The concentration of elements in leaves (mg kg^{-1} DW)				
	Total Fe	Active Fe	Mn	Zn	Cu
1	142 a	50 a	7.6 c	25 b	4.6 c
2	94 c	46 a	5.9 c	22 bc	7.1 b
3	145 a	36 b	7.4 c	29 ab	3.8 c
4	149 a	43 bc	10 b	26 b	11.9 a
5	119 b	18 d	12.9 b	31 a	9 ab
6	97 c	33 b	14.7 b	20 c	4.9 c
7	147 a	46 a	34.9 a	19 c	6.9 b
average	127	38.85	13.34	24.57	6.88

Table 5. The concentration of microelements in Miyagawa satsuma mandarin on Swingle citrumelo rootstock in different soils. (Mean values for each column with the same letters are not significantly different at 5% probability level).

Soils	The concentration of elements in roots (mg kg^{-1} DW)				
	Total Fe	Active Fe	Mn	Zn	Cu
1	1118 a	43 b	47 ab	64 d	15.3 d
2	1121 a	37 b	48 ab	114 b	39.3 a
3	836 c	40 b	38 bc	108 bc	20.2 c
4	837 c	41 b	34 c	102 c	31.2 b
5	914 b	61 a	41 b	161 a	32.9 b
6	830 c	22 c	39 bc	97 c	20.2 c
7	967 b	42 b	52 a	75 d	19.8 c
average	946	40.8	42.71	103	25.53

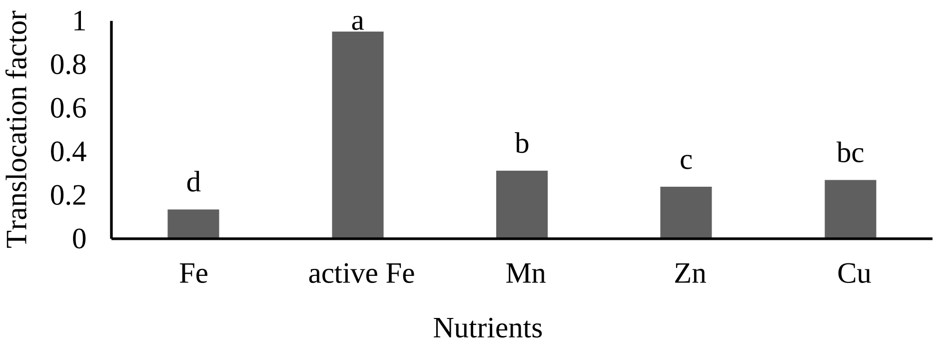

Fig. 5. Translocation factor of microelements in Miyagawa satsuma mandarin on Swingle citrumelo rootstock. (Mean values with the same letters are not significantly different at 5% probability level).

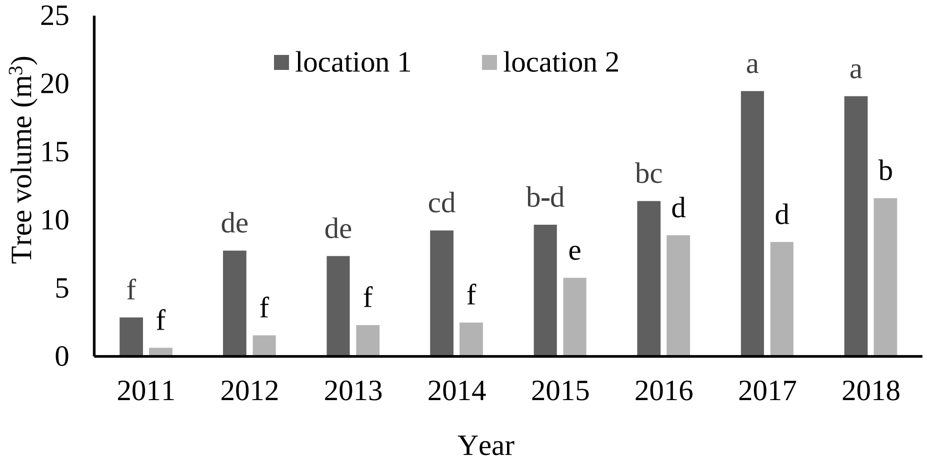

Fig. 6. Tree volume of Miyagawa satsuma mandarin on Swingle citrumelo rootstock in two locations during eight years. (Mean values with the same letters are not significantly different at 5% probability level)

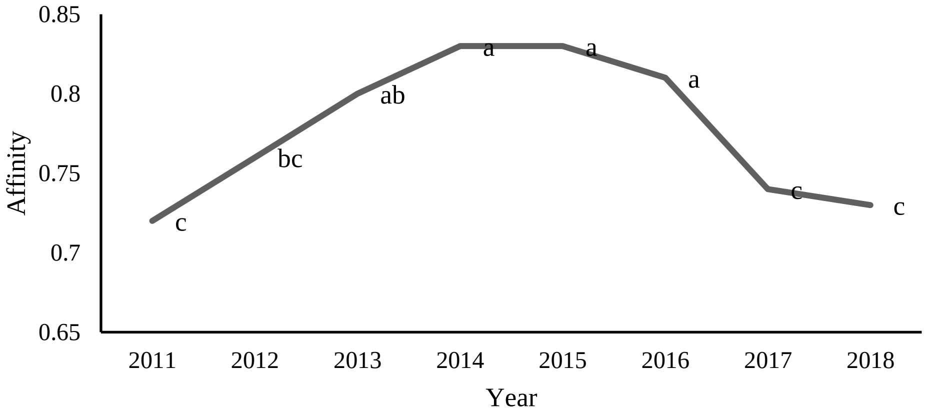

Fig. 7. Trunk affinity of Miyagawa satsuma mandarin and Swingle citrumelo during eight years. (Mean values with the same letters are not significantly different at 5% probability level)

Fruit analysis during three consecutive years (2014-2016) in the two locations showed that at the beginning of the reproductive stage, fruits were larger and their peel was thicker. By gradually going through the transition phase, the fruits became smaller, juicier, sweeter, and the peel became thinner and a brighter green. Also, ascorbic acid content increased (Table 6). The average fruit weight of Miyagawa mandarin on Swingle citrumelo rootstock during three years, in both locations, was 123 g in September and 139 g in October. Fruit juice was 42% in September and 44% in October. Peel percentage was 48.1% in September and 47.3% in October. Peel thickness was 3.04 mm in September and 2.7 mm in October. The harvest index was 4.91 in September (data not shown) and 8.46 in October (Table 6). The fruit size in different years in location 1 was greater than the fruit size in location 2. Fruits in location 2 always had a thicker and heavier peel. Total soluble solids (TSS), total acidity (TA), and ascorbic acid (AA) in piedmont (location 2) were significantly higher than those in the plain (location 1). Harvest index (TSS/TA) during three consecutive years in mid-September in location 1 varied from 5 to 5.5 and in location 2 varied from 3.9 to 3.5 (data not shown).

Table 7 shows the tree-producing indexes in the two locations during 5 years (2014-2018) in the first reproductive stage of trees. The yield efficiency of trees in location 2 was higher than in location 1 (6.28 and 4.62 kg m^{-3}, respectively) due to the smaller volume of trees in location 2. In this experiment, the average yield in the last two years was about 31 tons per hectare in both locations (500 trees ha^{-1}).

Table 6. Quality characteristics of Miyagawa satsuma mandarin on Swingle citrumelo rootstock in plain and piedmont (October 2014-2016). (Mean values for each column with the same letters are not significantly different at 5% probability level).

		Fruit weight g		Juice %		Peel weight %		Peel thickness mm		TSS %	TA %	TSS/TA	A.A. mg 100g^{-1}
		Sep.10	Oct.1	Sep.10	Oct.1	Sep.10	Oct.1	Sep.10	Oct.1	Oct.1	Oc.1	Oct.1	Oct.1
Loc.	L1	125.9	142.5	43.9a	45.0	46.11b	45.3b	2.59 b	2.5b	8.21 b	0.95b	9.28 a	53.83b
	L2	120.2	134.9	40.1b	42.5	50.05a	49.3a	3.48 a	2.9a	10.29a	1.54a	7.64 b	69.23a
	P. 5%	ns	ns	**	ns	*	*	**	**	**	**	**	**
Year	Y1	140.9a	149.4a	42.7b	43.2 b	49.35 a	48.9a	3.29a	2.9 a	9.38 ab	1.49a	6.58 b	53.68b
	Y2	124.4b	154.4a	37.4 c	41.0 b	50.57 a	47.0a	3.20a	2.7ab	8.63 b	1.43a	6.34 b	51.26b
	Y3	103.8c	112.4b	45.9 a	47.1 a	44.31 b	45.9a	2.62b	2.5 b	9.75 a	0.80b	12.45a	79.64a
	P. 5%	**	**	**	**	**	*	**	*	*	**	**	**
Year × Location	Y1L1	120.7bc	118.4b	47.3a	45.7ab	44.6 c	46.2ab	2.3c	2.7ab	9.25bc	1.23b	7.61b	50.16d
	Y1L2	161.0 a	180.4a	38.2b	40.7 b	54.06 a	51.6 a	4.3 a	3.2a	9.50a-c	1.74a	5.55c	57.20c
	Y2L1	140.4 b	177.6a	37.4b	40.8 b	49.92ab	45.4ab	2.8 c	2.7ab	6.88d	0.95c	7.31b	40.92e
	Y2L2	108.5cd	131.2b	37.3b	41.1 b	51.22 a	48.7ab	3. 6b	2.7ab	10.38ab	1.92a	5.38c	61.60c
	Y3L1	116.6 c	131.7b	47.0 a	48.5 a	43.76 c	44.4 b	2.6c	2.2 b	8.50c	0.66d	12.91a	70.40b
	Y3L2	91.0d	93.2c	44.7 a	45.8ab	44.87bc	47.5ab	2.6c	2.8 a	11.00 a	0.95c	11.99a	88.88a
	P. 5%	**	**	*	*	*	*	**	*	*	**	ns	**

Table 7. Vegetative and reproductive characteristics of Miyagawa satsuma mandarin trees on Swingle citrumelo rootstock in plain and piedmont settings (2014-2018). (Mean values in each column with the same letters are not significantly different at 5% probability level)

		Yield (kg tree^{-1})	Yield efficiency (kg m^{-3})	Cumulative yield (kg tree^{-1})	Alternate bearing Index (%)	Theoretical space (m^2 tree^{-1})	Theoretical number	Theoretical yield (ton ha^{-1})
Loc.	L1 (plain)	53.71	4.62 b	148.62 a	0.233 b	13.46 a	795 b	43.70
	L2(piedmont)	46.84	6.28 a	104.58 b	0.668 a	8.71 b	1421 a	49.22
	Prob. (5%)	ns	*	**	**	**	**	ns
Year	2014(Y1)	23.18 d	2.76 b	23.18 e	---	8.26 d	1692 a	20.64 d
	2015 (Y2)	37.74 c	7.21 a	60.92 d	0.552 a	9.37 c	1234 b	47.09 b
	2016 (Y3)	66.19 b	7.33 a	126.6 c	0.445 b	10.66 b	993 c	63.85 a
	2017 (Y4)	44.81 c	3.44 b	171.4 b	0.409 b	13.11 a	866 cd	33.12 c
	2018 (Y5)	79.45 a	6.52 a	250.9 a	0.398 b	14.03 a	755 d	67.59 a
	Prob. (5%)	**	**	**	**	**	**	**
Year × Location	Y1L1	45.36 cd	4.94 cd	45.36 ef	---	11.64 b	892 cd	38.79 d-f
	Y1L2	1.02 e	0.59 e	1.02 g	---	4.87 f	2493 a	2.49 g
	Y2L1	37.35 d	4.00 d	82.71 de	0.231 c	11.17 b-d	937 cd	33.78 f
	Y2L2	38.13 d	10.43 a	39.13 fg	0.873 a	7.57 e	1530 b	60.40 bc
	Y3L1	60.56 bc	6.02 b-d	143.3 c	0.251 c	11.51 bc	914 cd	53.05 c-e
	Y3L2	71.81 b	8.65 ab	109.9 cd	0.638 b	9.79 cd	1071 c	74.66 ab
	Y4L1	59.94 bc	3.24 de	203.2 b	0.233 c	16.50 a	619 d	36.99 ef
	Y4L2	29.69 d	3.63 de	139.6 c	0.585 b	9.72 d	1114 c	29.25 f
	Y5L1	65.34 b	4.91 cd	268.6 a	0.217 c	16.47 a	615 d	55.90 cd
	Y5L2	93.56 a	8.13 a-c	233.2 ab	0.578 b	11.60 b	896 cd	79.29 a
	Prob. (5%)	**	**	*	**	**	**	**

Discussion

The results of the potting experiment showed that the highest shoot dry weight and stem diameter of Miyagawa satsuma mandarin trees on Swingle citrumelo rootstock were obtained from soils with 14% and 2% lime and less than 9% calcium carbonate. According to the results, in the soils with more than 14% total lime and 5% active lime, the vegetative growth and shoot dry weight showed a sharp decrease. However, the lowest leaf chlorosis was obtained from soils with 3 and 9% total lime, whereas soils with 14 and 16% active lime caused the most severe chlorosis. The concentration of Fe in the roots was 7.45 times higher than in the leaves. In soils with the highest chlorosis degree, the relative Fe ratio of root to leaf was lower. Therefore, measuring the amount of available Fe in the soil (DTPA method) is not a good criterion for predicting the degree of leaf chlorosis in citrus trees or at least in this rootstock and scion. Fe deficiency in early growth, when spring shoots appear, leads to slower growth of new leaves and reduces the leaf size, but if Fe deficiency occurs during leaf development, it reduces leaf chlorophyll concentration and causes leaf chlorosis. Thus, in calcareous soils, the small size of leaves is one of the symptoms of Fe deficiency (Kosegarten et al., 1999; Castle and Nunnallee, 2009).

The leaf Mn concentration in all experimental soils was less than optimal, which is consistent with the results of Asadi and Mahmoudi (2000) who reported that more than 90% of citrus in the north of Iran has a hidden or obvious deficiency of Mn. The results also showed that although the amount of available Mn in all experimental soils was more than optimal, the concentration of Mn in the leaves was less than optimal. A similar condition was reported in field studies on citrus cultivated soils (Asadi, 2019). The results showed that the Mn concentration in roots is much higher than that in the leaves (3.2 times). Some reports have shown that Mn deficiency is often in shallow soils with high organic matter on top of calcareous soils (e.g. some soils in the forest areas on the slopes of hills). Similarly, Mn deficiency was observed in sedimentary clay and silty soils, as well as in swampy soils with high amounts of lime, and in calcareous soils with high organic matter and poor drainage. In addition to the effect of calcareousness, salinity/sodicity, and low soil moisture content drastically reduced the availability of Mn in soils. A higher availability of Mn in soils could induce Fe-deficiency (Srivastava et al., 2008). The results of our study also showed no significant relationship between Mn concentration in the roots and leaves and, likewise, between soil properties such as total and active lime and Mn concentrations in the leaves.

The results showed that the Zn concentrations in the soils of the north of Iran were less than optimal, which is consistent with the results of Asadi and Mahmoudi (2000) who reported symptoms of Zn deficiency in 10 to 15% of citrus orchards, while Zn concentrations were below optimum in more than 50% of orchards. Various reports have shown that an increase in soil lime and pH caused a decrease in plant-availability of Zn, which is mainly due to an increase in the uptake of Zn by soil components, Zn deposition in the form of insoluble compounds and also reduced Zn transfer from the soil solution to the root surface, thereby reducing the diffusion coefficient of Zn. Soil organic matter may also exacerbate or reduce Zn deficiency because the reaction of Zn with organic acids, amino acids, and fluvic acid forms low-molecular-weight dissolved organic Zn complexes that can increase the availability of Zn in the soil. In contrast, the reaction of Zn with humic acids and humine resulted in the formation of high molecular weight complexes that are usually low in solubility or are insoluble and have very low availability. The availability of Zn in the soil is adversely affected by soil calcareousness, high P content, salinity/sodicity and over-liming, etc (Srivastava, 2012). The results also showed a significant, positive relationship between Zn and Cu concentrations in roots and leaves, but showed no significant relationship between soil properties such as total lime and active lime with Zn and Cu in the leaves (Asadi, 2021).

The present study showed that in calcareous soils, the availability of Fe in the soil and the insufficient concentration of Fe in the soil solution reduced Fe uptake by roots. However, Fe uptake from the soil by roots and Fe transferring from roots to the shoots are not the main cause of Fe deficiency. Since Fe deficiency occurs in cellular apoplasts of the leaves and roots, followed by a decrease in its physiological efficiency, only 0.42% of root Fe and 30% of leaf Fe were active.

There was no significant regression relationship between soil Mn availability and its concentration in the roots and leaves. Also, there was no significant relationship between Mn concentration in the roots and its concentration in the leaves. According to the results of this study, Mn deficiency in the leaves of citrus trees in the north of the country is not due to Mn deficiency in the soil but due to the plant's inability of transfer of Mn from the roots to the shoots. Despite the high Mn in the soil and roots, the leaf Mn concentration was deficient (Asadi, 2019). There was no significant relationship between plant-available Cu in the soil and their concentration in the roots and leaves. However, there was a significant positive relationship between these elements in the roots and in their concentration in the leaf. According to the results, it is recommended not to use Swingle citrumelo rootstock in soils with more than 14% total lime and 5% active lime.

To predict plant-available Fe in calcareous soils, sequential Fe extraction is recommended to study the distribution of different forms of Fe (soluble + exchangeable Fe, carbonate, bonded with organic matter in different soils. In calcareous soils with poor drainage, due to the slower decomposition of organic carbon, more organic matter usually accumulates, which can affect the plant's available Fe. Therefore, the use of sequential extraction and determination of chemical forms of Fe in the soil and the relationship of these forms with leaf active Fe may be an effective method for predicting the chlorosis and plant available Fe (Asadi and Akhlaghi, 2015). In general, most of the current nutrient diagnostic tools are meant to address the nutritional deficits of the next season's crop, instead of addressing the nutrient constraints in the current standing crop. In this regard, biochemical markers (metalloenzymes) aided diagnosis, but due to highly technical and sensitive methods, this method is not so widely practiced. Identifying nutrient constraints in citrus based on the morphological changes, as phenotypic expression, is so time-consuming that an orchardist is left with no option to attend to the deficiency symptoms in a standing crop. In the years to come, unless an early warning system in form of some nutrient probe or sensor may be developed, addressing nutrient constraints in the current standing crop will not succeed in concrete terms. The non-destructive method of nutrient stress diagnosis using hyper spectral analysis for proximal sensing can be a good option for the future (Srivastava, 2013).

Several studies have shown that fluctuations in day and night temperature lead to better fruit color and sugar accumulation. Color changes are also dependent on soil temperature fluctuations (Mesejo et al., 2012; Lado et al., 2018). These cases of research confirm the current results. In the piedmont setting (location 2), the temperature at night always was lower than in the plain (Mahdavi et al., 2016). Thus, temperature fluctuations were higher and soil temperature was lower in location 2. So the fruits of location 2 had more TSS throughout the three years (Table 6). According to the acceptable amount of harvest index in Iran (Akhlaghi, 2020), mid-September is not suitable for the beginning of harvest in Miyagawa satsuma mandarin on Swingle citrumelo rootstock in the north of Iran. The variation range of TSS/TA in consecutive years in early October was much more than in mid-September, but in location 2 it was always lower than in location 1 (Table 6). Therefore, the best time to start harvesting is in October and, generally, it occurs sooner in the plain than in the piedmont.

Cold weather is the most important climatic limitation for citrus. Despite the occurrence of several frosts during the 10-year experiment, none of the trees were destroyed. Studies have shown that Swingle citrumelo has good resistance to cold stress (Castle and Stover, 2001; Akhlaghi and Asadi, 2022) which is consistent with the results of this study. However, cold weather and freezing in February 2014 mostly damaged the trees in the piedmont (location 2), so the average yield in 2014 was very low. But in the same year, the average tree yield of the plain (location 1) was 45 kg (Table 7). This initial stress caused the trees of location 2 to enter an alternate-bearing cycle. Alternate bearing index during the 5 years (2014-2018) in trees of location 2 was 0.67 and in location 1 was 0.23. The threshold for the alternate-bearing cycle is 0.50, and the very large and very small fruit in location 2 was also due to this cycle (Monselise and Goldschmidt, 1982). Monselise and Goldschmidt (1982) reported that environmental stress can be a major reason for starting this cycle. Environmental variables, especially temperature, are key factors that affect plant growth, development, and productivity. Differences in the development, yield, and quality of fruit attributes in varying locations might be due to the different climatic conditions that are based on the temperature prevailing during the crop life cycle (Edriss et al., 2019).

For optimal land use, the planting distance of trees should be considered based on the expected canopy volume of the mature tree on the selected rootstock in the soil and climate of every region. A denser garden may provide faster and more economic returns despite higher initial investment. In the present research, the required theoretical area for each tree according to the tree height and canopy diameter (Tazima et al., 2013) in the last year of the experiment (2018) was 16.5 m^2 for location 1 and 11.5 m^2 for location 2 (Table 7). Thus, the number of trees could be increased based on the obtained canopy volume and horticultural management, including training young trees in the first 3-4 years after planting, as well as annual winter pruning in mature trees (Akhlaghi, 2019).

Conclusion

The results of the present research showed that the fastest growth of Miyagawa satsuma mandarin on Swingle citrumelo rootstock was obtained in soils with 14% lime. By increasing lime in the soil, vegetative growth gradually decreased. In soils with about 9% lime, there were no signs of chlorosis in the leaves and, by having more lime in the soil, the symptoms of chlorosis gradually increased. In soils with 30% lime, the highest degree of yellowing appeared. There was a significant negative regression relationship between the amount of active lime in the soil and the concentration of active Fe in the leaves. Therefore, this rootstock can be recommended in soils with less than 14% of total lime or with less than 5% of active lime. The field experiment showed tree volume in the plain was always higher than in the piedmont, but the affinity of scion and rootstock did not have any significant difference in the two locations. TSS, TA, AA of fruits were significantly higher in the piedmont. Also, the trees of the piedmont had alternate bearing cycles. The yield increased from 23 kg per tree at the beginning of the reproductive period to 80 kg in the final year of the experiment. The best recommendable harvest time of the fruit in this cultivar and rootstock is early October.

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Conflict
The authors indicate no conflict of interest for this work.

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