INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–125/AWB/2010/12–1–153–156
http://www.fspublishers.org

Short Communication

Cold Hardiness of Different Apple Rootstock Clones

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ABSTRACT

The purpose of this investigation was to report the effect of the extreme weather event of the past winter (2008) on different apple rootstock clones including B9, M9, MM106, M26 (exotic) and Azayesh (native) grown in different soil textures in Karaj region, Iran. Young plants obtained from rooted cuttings were grown individually in plastic pots (40 cm in diameter & 42 cm in height), filled with sand, manure fertilizer and different soil particles (1:1:1). Statistical analysis of data indicated significant differences between years for all of the studied parameters among rootstocks (except for leaf phosphorus content), different soil textures (except for leaf N, Ca & chlorophyll content, SPAD value, leaf surface, height & trunk diameter) and the interactive effects of rootstock × soil texture (except for trunk diameter, leaf Ca & P content, leaf chlorophyll & SPAD value). Field observations on the effects of extreme air temperatures (-4.7 vs. -0.5ºC) and different soil textures revealed that the studied rootstocks were different in their cold hardiness. Azayesh grown in a soil texture of silt 48%, sand 20%, clay 31% and lime 14% and M9 grown in silt 49.2%, sand 19.8%, clay 31% and lime 18% were the most tolerant rootstocks, respectively.

Key Words: Apple; Cold injury; Rootstock clones; Soil texture; Lime

INTRODUCTION

Extremely cold temperatures affected much of the Middle East region and the Central Asian countries during the first weeks of January 2008. The severe cold conditions brought sub-zero temperatures and Kazakhstan experienced low temperatures of -25°C and its neighboring Uzbekistan, having its lowest temperatures in nearly 4 decades. Snowfall in parts of the Middle East and Iran was the heaviest in more than a decade, prompting numerous avalanches and causing multiple traffic accidents. Parts of Iran had almost 55 cm of snow from January 4-6. In Baghdad, Iraq, snow fell for the first time in living memory on January 11. In Tajikistan, Uzbekistan and Afghanistan low temperatures during late fall and early winter were the main factors limiting food production in these and adjoining areas.

Karaj in Iran is located in 50.58 longitudes and 35.48 latitudes and has a semi-arid climate with an average annual precipitation of 293.8 mm, relative humidity of 50% and average temperature of 14.3°C. The average temperature during the first month of winter 2008 dropped to -4.7°C, while it was -0.5°C in January 2007 and its long-term record for this month is 2.3°C. These extreme weather conditions caused significant winter injury to different orchards in the region including different clones of apple rootstocks. Several studies have shown that dwarfing rootstocks are more winter hardy (Granger *et al.*, 1991; Quamme & Brownlee, 1997; Bite & Drudze, 2000). The works of these researchers on cold hardiness evaluation of apple rootstocks showed that M9, M27 and B9 were more cold tolerant than others. The purpose of this article was to report the effects of pre-said extreme weather events on cold hardiness of different apple rootstock clones including B9, M9, MM106, M26 and Azayesh (native) grown in different soil textures.

MATERIALS AND METHODS

This experiment was conducted at the Department of Horticulture, Seed and Plant Improvement Research Institute, Kara, Iran during 2007-2008. The experiment was set up as a split plot based on randomized complete block design with 25 treatments and 3 replications (75 experimental units). The exotic apple rootstock clones (M9, MM106, M26, B9) and a native rootstock (Azayesh) were randomized to the main plot units and 5 different soil textures were randomized to sub plots. Young plants obtained from rooted cuttings were grown individually in plastic pots (40 cm in diameter & 42 cm in height), filled with sand, manure and soil particles. The soil particles differed in their textures and percentages of lime level as shown in (Table I).

Effects of mean January air temperature during 2007 and 2008 winters were evaluated on leaf parameters (in summer) and vegetative vigor (in autumn). Stress

To cite this paper: Mirabdolbaghi, M., R. Zarghami and A.V. Azghandi, 2010. Cold hardiness of different apple rootstock clones. *Int. J. Agric. Biol.*, 12: 153–156

susceptibility indexes of cold injury were also calculated using the formula:

$$SSI = \frac{1-(Ys/YP)}{(\bar{Y}s/\bar{Y}P)}$$

Where (Ys) is the value of studied character for treatment in stress condition, (YP) the value of studied character for treatment in normal condition, ($\bar{Y}s$) the mean value of total treatments in stress condition and ($\bar{Y}P$) the mean value of total treatments in normal condition (Fernandez, 1992). The obtained data were statistically analyzed using SAS program (version 6.12).

RESULTS

The combined analysis of variances indicated that there were significant differences between years 2007 and 2008 for most of the studied parameters, among rootstocks (except for leaf phosphorous content), among different soil textures (except for leaf nitrogen, leaf surface, plant height & trunk diameter) (Table II). The interactive effects between rootstocks and soil textures were also significant for all of the studied parameters (except for trunk diameter, leaf N & P).

Field observations revealed that dropping mean air temperatures from -0.5°C to-4.7°C significantly affected the response of rootstocks to cold temperatures. Soil textures also had a significant influence on their responses. Grouping rootstocks grown in different soil textures for leaf parameters and vegetative vigor based on SSI revealed that rootstocks were different in their cold hardiness (Table III). Although B9 rootstock clone showed the most sensitive leaf surface SSI at all of the studied soil textures but also had the most tolerant SSI of leaf macro elements (N, P, K, Ca, Mg) in soil texture treatments 4 (silt 45%, sand 21%, clay 34% & lime 22%) and 5 (silt 41.4%, sand 21.6%, clay 37% & lime 26%). The rootstocks MM106, M9, M26 and Azayesh had the lowest SSI in leaf macro-element in all of soil textures. Azayesh grown in a soil texture of silt 48%, sand 20%, clay 31% and lime 14% and M9 grown in silt 49.2%, sand 19.8%, clay 31% and lime 18% were appeared to be the most cold tolerant rootstocks regarding at all of the tested indices, respectively.

DISCUSSION

Cold hardiness is important for winter survival of perennial plants. Therefore there has been considerable interest on developing methods for determining the level of plant cold hardiness. Most of these methods are based on controlled freezing tests followed by evaluation of freezing injuries. However, the choice of evaluation method depends on the aims of the study, the type and the physiological state of plant material tested and the available facilities. Visual observation is a method that can be used in parallel to confirm the results. For instant, on a macro level, the loss of cell membrane integrity may also be expressed as a soft, water-soaked appearance (Lindén, 2002). The visual method requires minimum of instrumentation and is considered reliable, though subjective and qualitative in nature (Stergios & Howell, 1973; Harrison *et al.*, 1978; Holubowicz *et al.*, 1982). The extreme weather events in 2007 and 2008, regardless of their unfortunates, provided an outstanding opportunity for us like many other researchers to easily evaluate the cold hardiness of our plant materials by visual method.

Regarding genotypic differences of cold hardiness among studied apple rootstocks, the present study confirmed that the effect of extreme weather events varied significantly among different rootstock clones grown in different soil

Table I: Different soil textures used for growing rootstocks

Soil treatment	Silt (%)	Sand (%)	Clay (%)	Lime (%)	Organic (%)	pH	EC (dS m^{-1})	N (%)	P (ppm)	K (ppm)
Soil texture 1	47.5	21.5	31	10%	0.63	8	0.7	0.07	8	480
Soil texture 2	48	20	31	14%	0.74	8	0.6	0.10	12	360
Soil texture 3	49.2	19.8	31	18%	0.82	7.9	0.6	0.13	15	250
Soil texture 4	45	21	34	22%	0.42	8	0.6	0.05	8	150
Soil texture 5	41.4	21.6	37	26%	0.68	7.7	1.1	0.09	32.5	410

Table II: Combined analysis of variances for leaf parameters and vegetative vigor

		MS values															
S.O.V.	**df**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Year (Y)	1	1568.46**	0.40**	1.09**	0.39**	867.56**	0.72**	1.29**	7.51**	4.39**	0.78**	2010230**	3181.4**	1845**	886.4**	4239**	176**
Y*block	4	86.74$^{n.s}$	0.01$^{n.s}$	0.02$^{n.s}$	0.01*	20.13$^{n.s}$	0.003$^{n.s}$	0.002$^{n.s}$	0.001$^{n.s}$	0.02$^{n.s}$	0.01**	842.3*	79.2*	66.62**	38.31**	315.7*	0.54$^{n.s}$
Rootstock (R)	4	2191.58**	0.69**	1.81**	0.42**	540.21**	1.28**	0.05$^{n.s}$	1.98**	0.44$^{*.*}$	0.07**	28591.7**	446.8**	4086**	2276.66**	3052**	16.9**
Y*R	4	1029.24**	0.08**	0.27**	0.07**	13.04$^{n.s}$	0.06$^{n.s}$	0.09$^{n.s}$	0.21**	0.45**	0.02**	29547.1**	568.97**	1936**	583.4**	365.2*	9.99**
Y*block*R	16	136*	0.01$^{n.s}$	0.02$^{n.s}$	0.01$^{n.s}$	16.82$^{n.s}$	0.008$^{n.s}$	0.001$^{n.s}$	0.01$^{n.s}$	0.01$^{n.s}$	0.001$^{n.s}$	414.84$^{n.s}$	43.9*	11.81$^{n.s}$	14.82$^{n.s}$	180.2$^{n.s}$	0. 3$^{n.s}$
Soil texture (S)	4	37.04$^{n.s}$	0.25**	1.00$^{*.*}$	0.21**	20.61$^{n.s}$	0.43**	0.006**	1.12**	0.15**	0.01**	6869.23**	49.14*	310.5**	91.45**	116.6$^{n.s}$	0.7$^{n.s}$
R*S	16	304.14$^{n.s}$	0.31**	1.09**	0.29**	27.65*	0.37**	0.01$^{n.s}$	0.27**	0.11**	0.01**	8376.1**	139.27**	583.4**	165.73**	204.9*	0.9$^{n.s}$
Y*S	4	345.83**	0.03$^{n.s}$	0.12**	0.03**	38.46*	0.48**	0.01$^{n.s}$	0.09**	0.15**	0.01**	6871.3**	45.28$^{n.s}$	148.9**	237.2**	72.7$^{n.s}$	0.2$^{n.s}$
Y*R*S	16	164.66**	0.89**	0.44**	0.07**	32.87*	0.40**	0.01$^{n.s}$	0.13**	0.07**	0.01**	6076.5**	145.6**	154.3**	102.13**	231.13*	1.2$^{n.s}$
CV%		15.24	8.9	6.2	8.05	27.90	3.5	10.76	6.72	12.34	5.5	5.57	22.30	7.51	6.67	14.53	17.96

*Significant at Ps 0.05 **Significant at Ps 0.01 n.s Not significant

1= leaf chlorophyll (SPAD), 2= leaf chlorophyll a (mg/100 g leaf fresh weight), 3= leaf chlorophyll b (mg/100 g leaf fresh weight), 4= leaf chlorophyll a+b (mg/100 g leaf fresh weight), 5= leaf surface area (cm2), 6= leaf N (% DW), 7= leaf P (% DW), 8= leaf K (% DW), 9= leaf Ca (% DW), 10= leaf Mg (% DW), 11=leaf Fe (mg/kg DW), 12=leaf Zn (mg/kg DW), 13=leaf Mn (mg/kg DW), 14=leaf B (mg/kg DW), 15=height (cm) and 16=trunk diameter (cm)

Table III: Grouping rootstock clones according to SSI for studied characters

Treatment		Tolerant (0 - 0.75)					Semi-tolerant (0.75 - 1)					Semi-susceptible (1 - 1.25)					Susceptible(>1.25)				
Rootstock	**Soil texture**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
	1	-	-	-	-	0.82	-	-	0.93	-	-	-	-	-	1.04	-	1.28	1.98	-	-	-
	2	0.33	-	-	-	0.70	-	-	0.93	0.99	-	-	-	-	-	-	-	3.01	-	-	-
B9	3	0.36	-	-	-	-	-	-	0.96	-	-	-	-	-	1.13	-	-	1.69	-	-	1.20
	4	-	-	0.20	-	-	0.94	-	-	0.95	-	-	-	-	-	1.11	-	2.10	-	-	-
	5	0.41	-	0.11	-	-	-	-	-	-	0.98	-	-	-	1.09	-	-	1.62	-	-	-
	1	0.08	-	0.33	-	-	-	-	-	-	0.95	-	-	-	1.05	-	-	1.45	-	-	-
	2	-	0.57	0.25	-	-	-	-	-	-	0.99	-	-	-	1.13	-	2.95	-	-	-	-
MM106	3	-	-	0.28	-	-	-	0.77	-	-	-	1.06	-	-	1.05	1.16	-	-	-	-	-
	4	0.60	-	0.30	-	-	-	0.96	-	-	-	-	-	-	-	1.09	-	-	-	1.40	-
	5	-	0.67	0.25	1.15	-	-	-	-	-	-	-	-	-	-	--	1.36	-	-	-	1.88
	1	0.83	0.63	0.15	1.10	-	-	-	-	-	-	-	-	-	-	--	-	-	-	-	1.31
	2	-	-	0.18	1.10	-	-	-	-	-	-	-	1.05	-	-	1.05	3.54	-	-	-	-
M26	3	-	-	0.16	0.97	-	-	-	-	-	0.83	-	1.21	-	-	-	2	-	-	-	-
	4	-	-	0.14	0.91	-	-	-	-	-	-	-	1.22	-	-	1.12	2.12	-	-	-	-
	5	-	-	0.33	0.91	-	-	-	-	-	-	-	1.09	-	-	-	1.78	-	-	-	1.88
	1	0.01	-	0.19	0.84	0.61	-	-	-	-	-	-	1.04	-	-	-	-	-	-	-	-
	2	0.28	-	0.14	-	0.62	-	0.93	-	-	-	-	-	-	-	-	-	-	-	1.30	
M9	3	-	-	0.10	0.98	0.66	0.87	0.79	-	-	-	-	-	-	-	-	-	-	-	-	
	4	-	-	0.14	0.92	0.60	-	0.88	-	-	-	-	-	-	-	-	1.86	-	-	-	
	5	-	-	0.21	-	0.65	-	-	-	-	-	-	-	-	-	-	1.86	1.26	-	1.25	
	1	0.53	0.67	0.27	0.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.31
	2	0.01	0.53	0.24	0.68	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Azayesh	3	-	0.40	0.30	0.82	-	-	-	-	-	0.92	-	-	-	-	-	2.81	-	-	-	-
	4	-	0.11	029	-	-	-	-	-	-	0.97	-	-	-	1.04	-	1.11	-	-	-	-
	5	0.09	0.26	0.15	0.92	-	-	-	-	-	0.89	-	-	-	-	-	-	-	-	-	-

1= SSI of leaf chlorophyll (mg/100 g leaf fresh weight), 2= Leaf surface area (cm^2), 3= SSI of leaf macro elements (%DW), 4= SSI of leaf micro elements (mg/kg DW), 5= SI of vegetative vigor (cm)

Table IV: Mean comparison (absolute values) of studied parameters in different apple rootstocks in 2 years (2007 & 2008)

		Recorded parameters															
Rootstock	**Year**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
B9	2007	53.32	0.84	2.10	0.85	23.04	1.97	0.16	1.94	1.40	0.39	244	36.68	68.40	47	68	3.8
	2008	23.8	0.80	2.08	0.86	17.05	2.27	0.38	1.49	1.07	0.27	372.53	14.69	77.29	33.50	79.13	5.73
MM106	2007	48.56	0.86	2.29	0.89	14.63	2.29	0.18	2.46	1.67	0.45	241.70	29.58	73.29	51.30	81.93	3.2
	2008	22.2	0.94	2.28	0.87	11.35	2.35	0.52	1.94	0.92	0.29	482.58	18.45	41.13	38.23	94.13	5.2
M26	2007	66.51	1.10	2.54	1.07	16.86	2.30	0.20	2.35	1.14	0.46	205.80	17.72	61.40	51	80.8	4.23
	2008	38.78	1.28	2.84	1.22	11.55	2.4	0.36	1.67	0.82	0.33	441.61	17.44	48.64	29.13	90	8.13
M9	2007	30.01	0.74	1.95	0.76	16.85	2.12	0.16	1.90	1.23	0.42	214.90	19.29	41.89	62.10	63.07	5.13
	2008	28.78	0.97	2.36	1.01	10.89	2.23	0.34	1.51	1.06	0.23	514.03	17.44	43.51	38.20	64.13	5.8
Azayesh	2007	46.82	0.81	2.05	0.82	9.88	2.59	0.23	2.35	1.22	0.57	170.40	23.17	46.91	61	73.47	3.77
	2008	28.56	0.89	2.22	0.94	6.37	2.71	0.28	2.13	1.09	0.35	423.70	12.38	46.1	60.13	93.73	5.93

1= leaf chlorophyll (SPAD value),.2= leaf chlorophyll a (mg/100g leaf fresh weight), 3= leaf chlorophyll b (mg/100 g leaf fresh weight), 4= leaf chlorophyll a + b (mg/100 g leaf fresh weight), 5= leaf surface area (cm2), 6= leaf N (%DW), 7= leaf P (%DW), 8= leaf K (%DW), 9= leaf Ca (%DW), 10= leaf Mg (DW%), 11= leaf Fe (mg/kg DW), 12= leaf Zn (mg/ kg DW), 13= leaf Mn (mg/kg DW), 14= leaf B (mg/kg DW), 15= height (cm) and 16= trunk diameter (cm)

textures. Mean comparison of studied parameters (absolute values) among different rootstocks grown in different soil textures for the years 2007 and 2008 showed significant decreases for leaf parameter values recorded in summer 2008 (Table IV). In particular, leaf chlorophyll (SPAD) values of all rootstock clones deceased more considerably e.g., 62% for B9, 53% for MM106, 74% for M26, 98% for M9 and 76% for Azayesh (Table IV).

There are many factors affecting susceptibility of plant to winter injury. These include but not limited to cultivars, rootstocks, fall hardening, snow cover, air temperature, cultural practices, plant nutritional and its carbohydrates status (Robinson *et al.*, 2002; Cline *et al.*, 2005). Trees on dwarf rootstocks planted in sandy, sandy loam, gravel loam or any other types of soil combined with sand or gravel were more susceptible to winter damage. This might be due to the shallow root system of dwarf rootstock in sandy soils (Buszard, 1981).

In the present study, it was found that the different soil textures have significant influence on the response of rootstocks to cold injury (Table III). Based on SSI formula (Fernandez, 1992) and grouping rootstocks grown in different soil textures for their leaf parameters and vegetative vigor (Table III) it can be suggested that Azayesh and M9 clones grown in soil texture 2 (silt 48%, sand 20%, clay 31% & lime 14%) and 3 (silt 49.2%, sand 19.8%, clay

31% & lime 18%), respectively were the most tolerant rootstocks. Finding M9 as a winter hardy rootstock is in agreement with Cline *et al.* (2003 & 2005). Interestingly the native apple rootstock, Azayesh with its genetic diversity studied by Kianamiri *et al.* (2007) showed relatively high winter hardiness.

In conclusion, the extreme weather events happened during winter 2008 provided us a useful mean for measuring cold hardiness characteristics of some apple rootstock clones and determination the impact of different soil textures on the level of hardiness experimentally. Based on the observations, Azayesh and M9 clones showed greater winter hardiness than M.M106, M.26, and B.9, which can be cultivated in more frost affected areas.

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(Received 20 April 2009; Accepted 13 May 2009)