Tree and Root Architecture of *Malus sieversii* Seedlings for Rootstock Breeding

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Abstract

The foundation of a successful apple orchard is in large part the rootstock used to establish the trees in that orchard. Apple rootstocks can impart several important architectural tree characters to the scion, among which are reduction in tree size and early production of flowers/fruit. It is probable that similar root-mediated characteristics exist in natural ancestral apple populations such as *Malus sieversii*, a species known to have traits associated with tolerance to several biotic and abiotic stresses. We sought to understand the genetic determinism of tree architecture of *M. sieversii* seedlings by measuring several scion and root architecture characters on a total of 1,180 high resolution images of dormant 1-year-old trees. These images were analyzed to ascertain number of growing points (tips), tree volume and total length of branch canopy, flat branching, presence of spines, root mass, number of primary roots, and number of thick roots. Analysis of means revealed significant inherited differences for several traits related to tree and root architecture, especially for flat branching, presence of spines, number of primary roots and root mass. Such differences were also detected among sites of origin of the mother trees. We have used this data to select parents for a new generation of rootstocks that will be evaluated in years to come.

INTRODUCTION

The success of modern apple orchards is dependent on specific types of tree architecture inherent to varied apple genotypes or imparted by the use of certain rootstocks. Tree size and shape, sylleptic and proleptic growth, columnar habit, spines and the development of fruiting spurs are examples of inherent characteristics (Lauri et al., 2011). Additionally, tree size, branch angles, branching and percentage of fruiting wood are examples of architectural traits that can be modified by specialized rootstock genotypes (Fazio and Robinson, 2008). The ability of rootstocks to modify key architectural features of scions is heritable and is currently derived from a very restricted germplasm pool (Malling series of rootstocks and derivatives). These rootstocks are susceptible to some biotic and abiotic stresses, and these weaknesses are costly to the apple industry (Russo et al., 2007). It is likely that similar root-imparted characteristics exist in natural apple populations of *Malus sieversii*, a species that also has traits associated with tolerance to several biotic and abiotic stresses (Fazio et al., 2009). Individual *M. sieversii* trees could have genetic factors that mimic or improve the positive dwarfing and precocity traits that are found in Malling germplasm. In addition, *M. sieversii* germplasm might contribute characteristics such as deep root exploration, drought tolerance or resistance to diseases and insects to existing rootstocks, thus creating more productive, and ecologically and economically sustainable rootstocks. In this manuscript, we describe efforts to identify germplasm for rootstock breeding in the *M. sieversii* gene pool on the basis of tree architecture.

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MATERIALS AND METHODS

We used a series of genetically related seedlings derived from a project aimed at preserving the genetic diversity of *M. sieversii* through the development of a seed core collection to evaluate tree architecture traits (Richards et al., 2009; Volk et al., 2005). This seed core collection was generated by inter-mating a set of trees representing a very high level of genetic diversity for the species. Several sets of flowers from each mother tree were pollinated by bulked pollen from specific sets of other mother trees from the core set, so that each tree in the core set was the donor of both megaspores and microspores (Table 1). In some cases, the core individuals that were selected had a sib relationship (e.g., 3610.b and 3610.l were both seedlings collected from the same seed-lot/mother-tree #3610 collected in site 9 in Kazakhstan). Approximately, 500 seeds from Kazakhstan site 6 and site 9 core individuals were germinated and planted in the McCarthy nursery of the USDA ARS PGRU repository in spring of 2008 and allowed to grow for two seasons. Surviving seedlings were harvested in the fall of 2009 and two high resolution TIFF images (the second one with tree rotated 90°) of each tree against a white backdrop were collected between December 2009 and March 2010 (samples displayed in Figures 1 and 2). A total of 1,180 high resolution images were then analyzed using the WinRhizo software resulting in data for average stem diameter, number of growing points (tips), number of bifurcations, tree volume and total length of branch canopy. The same images were then visually evaluated for tree architecture features such as flat branching, presence of spines, root mass, number of primary roots, and number of thick roots. Means and standard errors for half sib families and for bulked pollen pools were calculated using SAS JMP 10 (SAS Institute) statistical software and displayed in Table 2.

RESULTS AND DISCUSSION

The *M. sieversii* seedling trees examined in this study exhibited ample variation for all traits under evaluation. When grouped by mother tree, half sib individuals exhibited more similarity within family and less among half sib families (Figs. 1 and 2). Relative to the mother trees, the pollen pools did not seem to have a significant effect on all traits measured in this study, however there were significant differences detected among some traits on the basis of site of origin of the germplasm (Fig. 3). We expected no significant effects of the pollen pools on the measured phenotypes, since the pollen pools were composed of pollen from a number of heterogeneous individuals and each individual's heritable contribution is dampened (Table 1). Therefore, the effects (variance) caused by the alleles transferred to the progeny from the same mother tree is clearly identifiable and heritable. The means and standard errors found in Table 2, can be used to identify optimal source material for breeding on the basis of their seedling architecture. For example, individuals of families 3610 might be a good source for flat branching (indication of rootstock induced fruiting wood) and individuals from 3620 and 3674 might be useful to breed lack of spines (indication of lack of juvenility and desirable nursery trait). Also, noteworthy for the spine trait, is the fact that there is variation among some related families (4002 d, e, f, and l), while others are homogeneous (3781 b, c, and n). Correlation coefficients (data not shown) among traits were significant ($p<0.01$), but were generally below 0.5. Among the correlations between scion and root traits, the correlation between canopy volume/tree size and number of thick roots was 0.38 ($p<0.001$), while the correlation of tree size and root mass was less pronounced 0.25 ($p<0.001$), indicating that the vigor/size of the young trees was determined in part by their ability to produce root systems with strong primary hierarchy. While these root traits may be of use for increasing root anchorage in apple rootstocks, they may provide some undesirable effects on tree vigor. Flat branches were positively correlated with root mass with coefficient of 0.26 ($p<0.001$) and root branching with coefficient of 0.24 ($p<0.001$) in Site 9 mother trees, but not in Site 6. This observation is one of several that reflect marked differences in architecture phenotypes between mother trees on the basis of their origin site in Kazakhstan (Fig. 3). Site 9, on average ,had significantly smaller seedlings, flatter branches, higher number of thick and primary roots, fewer spines and larger root masses than Site 6. These differences may reflect site-specific pedo-climatological adaptations. Compared to Site 6, Site 9 had a higher elevation and received less precipitation (Forsline and Aldwinckle, 2004).

CONCLUSIONS

Although the initial purpose of these seedlings was intended for additional disease resistance characterization and to check the successful preservation of allelic diversity of *M. sieversii* by seed, the selected set of *M. sieversii* trees provided a unique opportunity to study the architectural phenotypic diversity of this species and identify genotypes with unique architectural characters for breeding new rootstocks. This analysis revealed substantial genotypic effects on tree and root architecture, especially for flat branching patterns, presence of spines, number of primary roots and root mass. It also revealed that perhaps natural adaptation to site-specific, pedo-climatic conditions has occurred in this species and such adaptations may be captured through breeding. This material is available through the USDA ARS Plant Genetic Resources Unit website for distribution to qualified members of the scientific community. In addition to disease resistance, *M. sieversii* is proving to be a good source for architectural characters linked to orchard productivity. As such, the data in this manuscript can be used to select mother trees as parents of future crosses as our breeding program has already done with elite Geneva® apple rootstocks.

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Tables

Table 1. Mother tree and pollen pool designations and *S-RNAse* alleles of each mother tree characterized according to Dreesen et al. (2010), for seedlings in this analysis.

Kazakhstan site	GMAL Num.	SDLG	Row	Tree	Pollen pool	S Allele 1*	S Allele 2
6	3682	.k	6	6	B	359	NA
6	3683	.i	6	15	B	345	NA
6	3683	.n	6	20	A	343	356
6	3684	.a	6	22	B	345	585
6	3684	.b	6	23	B	345	NA
6	3684	.l	6	33	B	345	NA
6	3685	.d	6	40	C	359	545
6	3685	.e	6	41	C	318	585
6	3685	.f	6	42	A	345	545
6	3687	.d	6	55	D	343	NA
6	3688	.n	7	20	B	365	545
6	3689	.c	7	24	A	365	NA
6	3689	.n	7	35	B	365	545
6	3690	.o	7	53	A	359	585
6	3691	.m	8	7	D	356	359
6	3975	.d	9	36	C	338	345
6	3975	.m	9	45	A	343	362
6	3989	.f	9	52	D	545	585
6	3989	.k	9	56	C	345	365
6	3999	.b	10	2	D	343	362
6	4000	.b	10	17	C	362	545
6	4002	.d	10	33	A	338	359
6	4002	.e	10	34	C	362	NA
6	4002	.h	10	37	A	362	NA
9	3608	.a	1	47	B	343	495
9	3608	.b`	1	48		338	362
9	3610	.b	2	4	D	343	362
9	3610	.l	2	14	D	345	362
9	3614	.a	2	15	A	338	359
9	3614	.g	2	21	A	338	359
9	3616	.d	2	30	B	343	345
9	3619	.j	2	51	B	338	495
9	3619	.m	2	54	B	372	NA
9	3620	.e	3	3	A	345	365
9	3620	.m	3	11	A	338	362
9	3623	.f	3	29	B	338	495
9	3627	.a	4	6	B	318	343
9	3627	.l	4	17	B	318	362
9	3629	.n	4	32	A	343	495
9	3638	.b	5	31	D	343	372
9	3762	.g	8	16	D	338	NA
9	3762	.n	8	23	B	372	585
9	3764	.e	8	29	D	372	NA
9	3764	.l	8	36	D	362	585
9	3781	.b	8	53	C	362	495
9	3781	.c	8	54	A	NA	NA
9	3781	.n	9	4	B	362	585
9	3785	.b	9	22	A	362	NA
9	4020	.i	11	33	C	NA	NA
9	4024	.n	11	49	C	NA	NA

* NA= Not amplified.

Table 2. Means (top) and standard errors (bottom) for several architecture descriptors measured on the seedling trees arranged by mother tree.

Mother tree	Flat branches	% Spines	Root mass	Root bran.	Thick roots	Prim. roots	Canopy length (cm)	Canopy volume (cm^3)	Number of apices
3608.a	6.32	0.55	4.27	2.45	5.18	5.18	644.91	70.22	85.00
	0.95	0.11	0.44	0.28	0.50	0.50	88.86	12.07	18.95
3610.b	10.89	0.48	5.00	4.74	9.30	8.44	585.45	86.66	71.30
	1.08	0.10	0.42	0.45	0.64	0.63	48.49	11.89	9.07
3610.l	7.73	0.62	3.23	4.62	11.23	8.31	1028.31	123.23	151.81
	1.04	0.10	0.39	0.46	0.96	0.67	106.62	14.37	22.22
3614.a	5.43	0.57	2.86	5.43	7.93	5.36	574.90	73.74	61.29
	1.07	0.14	0.54	0.80	0.49	0.75	46.29	9.91	10.83
3614.d	6.53	0.89	4.05	5.79	10.42	6.26	673.98	75.80	114.16
	0.91	0.07	0.36	0.57	0.86	0.55	45.53	10.35	12.36
3616.d	5.53	0.33	4.13	4.67	8.73	6.33	390.05	44.25	45.67
	0.90	0.13	0.66	0.69	0.91	1.00	43.17	6.58	6.03
3619.j	4.31	0.55	5.10	5.83	10.69	6.59	612.69	75.95	91.77
	0.78	0.09	0.41	0.37	1.17	0.49	69.79	9.80	15.27
3619.m	5.42	0.25	4.58	4.50	11.50	5.58	474.56	62.55	64.67
	1.33	0.13	0.47	0.51	0.95	0.72	89.99	10.30	22.42
3620.e	11.33	0.00	5.33	6.00	7.00	5.33	574.62	89.85	48.33
	2.03	0.00	0.33	0.00	3.79	0.67	31.68	4.01	3.28
3620.m	8.52	0.22	4.67	4.93	11.63	6.67	515.53	75.99	43.56
	1.19	0.08	0.33	0.46	0.75	0.52	32.10	7.00	4.86
3623.f	7.50	0.00	4.25	5.00	10.63	8.13	631.45	84.51	37.38
	1.48	0.00	0.41	0.71	0.56	1.17	80.79	10.33	5.73
3627.a	5.94	0.25	6.00	6.50	13.63	9.06	455.17	61.20	30.25
	1.30	0.11	0.61	0.62	0.99	0.86	51.87	6.56	4.59
3627.l	6.11	0.78	5.56	6.11	10.72	6.67	642.65	89.33	57.89
	1.30	0.10	0.44	0.53	0.87	0.60	54.37	14.70	4.98
3629.d	3.58	0.42	4.16	4.05	8.79	6.79	433.21	59.68	51.53
	1.29	0.12	0.30	0.46	0.60	0.55	65.69	9.25	8.59
3629.n	5.27	0.73	4.36	4.36	11.27	7.09	618.82	109.91	73.55
	0.73	0.10	0.38	0.44	0.53	0.57	38.24	16.88	7.44
3638.b	6.58	0.81	5.27	5.08	12.15	7.92	643.30	80.12	96.77
	1.18	0.08	0.39	0.51	1.22	0.71	57.35	9.32	11.37
3682.k	4.30	1.00	3.60	3.10	8.20	4.40	688.77	97.68	148.40
	1.20	0.00	0.34	0.28	0.49	0.27	79.74	12.14	26.79
3683.f	5.18	1.00	3.55	2.91	6.82	5.00	1021.22	105.63	159.45
	1.63	0.00	0.37	0.28	0.63	0.36	52.71	9.56	13.21
3683.i	3.09	0.73	3.05	3.09	7.45	5.64	638.55	86.06	91.73
	0.72	0.10	0.15	0.31	0.46	0.48	61.19	11.78	10.91
3683.n	3.41	0.91	3.82	4.55	7.09	6.00	723.45	74.18	153.95
	0.56	0.06	0.31	0.42	0.55	0.39	71.20	8.51	8.19
3684.a	5.73	0.54	4.46	5.88	8.42	6.92	767.85	87.08	103.85
	1.04	0.10	0.41	0.40	0.55	0.61	46.61	8.04	11.17
3684.b	3.62	0.81	5.95	6.67	10.71	4.90	711.25	75.06	122.38
	0.98	0.09	0.31	0.28	0.74	0.49	73.55	12.03	22.26
3684.l	6.53	0.73	3.87	4.20	8.50	5.10	691.66	82.35	85.40
	0.60	0.08	0.28	0.44	0.47	0.36	80.26	6.28	19.16

Mother tree	Flat branches	% Spines	Root mass	Root bran.	Thick roots	Prim. roots	Canopy length (cm)	Canopy volume (cm^3)	Number of apices
3685.d	2.65	0.80	3.20	4.70	6.85	5.00	714.92	89.84	115.35
	0.61	0.09	0.34	0.40	0.63	0.53	64.93	11.74	12.58
3685.e	1.50	1.00	5.00	3.00	9.00	4.75	784.83	78.79	92.25
	0.50	0.00	0.58	0.58	1.68	0.95	61.08	12.17	11.23
3685.f	3.41	0.94	4.12	4.71	9.06	4.82	724.56	68.82	122.18
	0.68	0.06	0.33	0.37	1.02	0.61	93.94	9.10	34.68
3687.d	2.30	0.40	4.00	4.30	7.85	5.25	740.79	83.82	107.35
	0.59	0.11	0.34	0.37	0.50	0.58	78.53	10.11	14.19
3688.n	2.25	1.00	3.80	4.10	8.90	4.40	756.16	82.48	116.65
	0.62	0.00	0.22	0.52	0.60	0.63	58.80	6.32	14.28
3689.c	2.64	0.71	2.57	4.57	6.79	5.64	768.48	122.27	80.29
	0.87	0.13	0.25	0.59	0.50	0.44	74.58	21.59	7.31
3689.n	2.10	0.80	5.80	6.20	9.25	6.45	558.81	69.96	85.30
	0.51	0.09	0.47	0.53	0.68	0.66	47.82	7.33	9.80
3690.o	2.50	0.50	3.00	2.50	9.00	3.00	700.74	79.38	122.50
	1.50	0.29	0.58	0.29	2.68	0.91	295.12	35.00	31.81
3691.f	0.75	0.83	4.92	3.92	8.67	7.04	591.36	98.82	74.67
	0.17	0.08	0.47	0.48	0.61	0.60	43.98	11.37	5.84
3691.m	3.36	0.71	4.29	4.93	9.00	4.79	790.08	74.08	106.38
	0.56	0.13	0.40	0.46	0.90	0.39	95.83	9.52	15.50
3762.g	4.46	0.92	4.92	5.75	11.21	7.83	659.85	81.86	108.38
	0.67	0.06	0.45	0.50	1.06	0.73	57.32	10.20	11.40
3762.h	3.13	0.60	4.70	5.10	10.53	6.47	594.78	72.95	67.20
	0.49	0.09	0.28	0.36	0.69	0.52	28.32	5.62	5.57
3762.n	5.29	0.60	3.76	3.05	9.48	3.52	623.34	74.49	103.62
	0.88	0.11	0.36	0.29	0.85	0.43	82.89	17.58	23.58
3764.e	3.64	0.54	4.21	3.50	9.25	5.39	749.53	85.68	85.86
	0.60	0.10	0.34	0.44	0.75	0.58	53.51	6.79	15.12
3764.l	3.81	0.58	3.73	3.62	9.58	4.12	706.75	70.95	99.19
	0.74	0.10	0.27	0.28	0.72	0.39	85.28	5.90	21.81
3775.h	5.44	0.60	3.68	3.60	9.40	4.56	513.32	68.42	64.56
	0.52	0.10	0.27	0.34	0.48	0.55	30.53	5.48	5.74
3781.b	1.89	0.70	4.33	4.78	9.30	5.63	585.99	76.14	79.81
	0.47	0.09	0.23	0.35	0.75	0.39	60.48	9.88	9.76
3781.c	4.88	0.68	4.16	4.44	10.04	4.60	720.55	80.44	104.48
	0.85	0.10	0.26	0.35	0.75	0.45	63.88	9.02	11.19
3781.n	3.62	0.69	4.31	5.45	8.66	6.66	584.79	84.98	107.34
	0.65	0.09	0.32	0.33	0.42	0.45	48.50	8.05	12.54
3785.b	2.50	1.00	3.93	3.29	9.29	5.93	721.22	95.93	116.14
	0.72	0.00	0.25	0.57	0.83	1.07	52.97	8.12	14.82
3975.d	1.00	0.75	4.13	4.00	10.19	4.81	721.79	106.95	91.56
	0.38	0.11	0.27	0.48	1.05	0.63	78.32	10.95	9.70
3975.m	3.04	0.58	4.50	4.75	8.67	7.00	577.73	88.46	55.88
	0.58	0.10	0.29	0.34	0.74	0.44	52.00	8.02	7.44
3989.f	2.56	0.56	3.26	3.26	8.04	4.19	559.54	75.51	65.52
	0.61	0.10	0.29	0.32	0.63	0.40	43.87	8.14	9.14
3989.k	3.45	0.68	3.14	5.32	7.55	4.77	677.70	82.14	94.27
	0.74	0.10	0.20	0.36	0.55	0.46	48.49	6.78	14.44

Mother tree	Flat branches	% Spines	Root mass	Root bran.	Thick roots	Prim. roots	Canopy length (cm)	Canopy volume (cm^3)	Number of apices
3999.b	3.44	0.88	2.69	2.69	6.31	5.44	718.61	79.25	107.88
	0.96	0.09	0.33	0.33	0.64	0.66	81.97	12.68	11.08
4000.b	2.97	0.86	3.62	3.59	8.62	5.45	656.78	93.97	118.34
	0.51	0.07	0.19	0.20	0.60	0.58	52.31	12.85	15.86
4002.d	3.54	0.79	3.50	3.64	9.39	5.29	669.43	72.10	92.61
	0.72	0.08	0.32	0.36	0.63	0.58	53.49	7.10	8.85
4002.e	5.91	0.82	2.55	3.18	8.27	4.00	663.04	77.32	72.91
	0.78	0.12	0.41	0.60	0.51	0.73	38.77	8.37	6.32
4002.f	1.00	1.00	3.00	4.00	6.13	2.63	766.42	86.73	116.00
	0.63	0.00	0.46	0.60	0.83	0.56	170.21	22.66	29.08
4002.h	1.84	0.58	3.13	4.65	8.39	6.26	506.69	56.72	88.52
	0.40	0.09	0.28	0.35	0.52	0.49	65.32	6.74	17.88
4002.l	4.24	0.56	2.96	2.84	8.84	4.60	518.81	81.33	60.32
	0.37	0.10	0.19	0.29	0.48	0.46	45.79	10.36	5.53
4020.i	1.50	0.70	4.10	3.70	8.35	7.00	465.11	50.52	76.00
	0.37	0.11	0.44	0.49	0.74	0.67	79.42	6.74	14.74
4024.l	2.58	0.89	4.32	3.95	10.37	6.16	492.60	62.97	72.26
	0.63	0.07	0.49	0.54	0.65	0.70	65.15	11.56	9.32
4024.n	2.92	0.67	2.17	2.17	6.75	3.92	544.01	75.92	57.67
	0.83	0.14	0.41	0.11	0.62	0.48	87.60	15.33	7.76
4331	4.80	0.40	4.16	3.44	8.68	4.96	525.08	100.87	78.84
	0.67	0.10	0.26	0.27	0.71	0.43	46.99	17.60	22.32
4446	4.60	0.40	4.73	5.07	7.20	6.67	494.52	98.11	69.53
	1.32	0.13	0.67	0.62	1.03	0.63	61.82	12.62	12.20

Figures

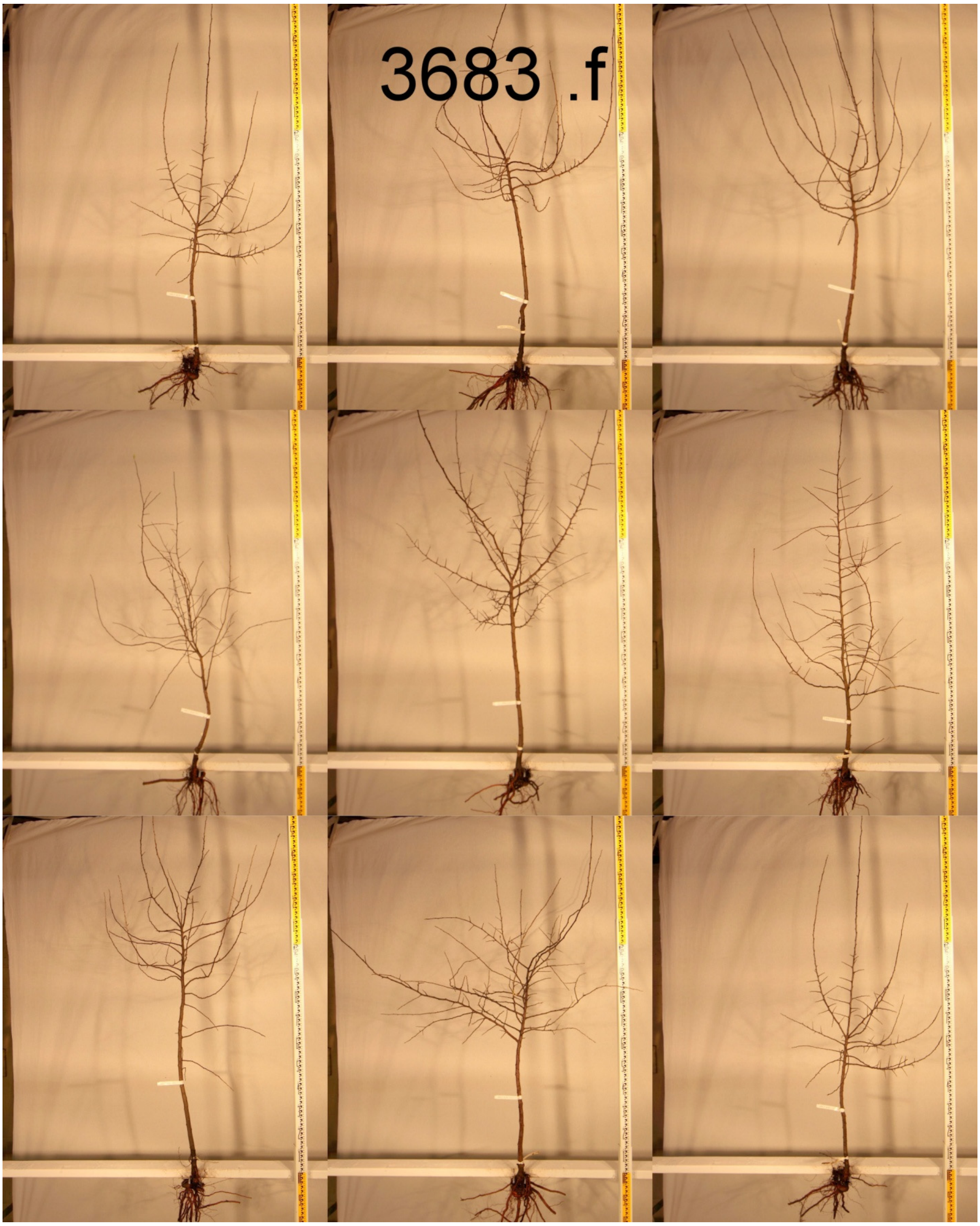

Fig. 1. Sampling of high resolution images of seedlings derived from the GMAL 3683.f mother tree. Although the group seems heterogeneous, there are some similarities in tree size, number of branches and general shape of the canopy.

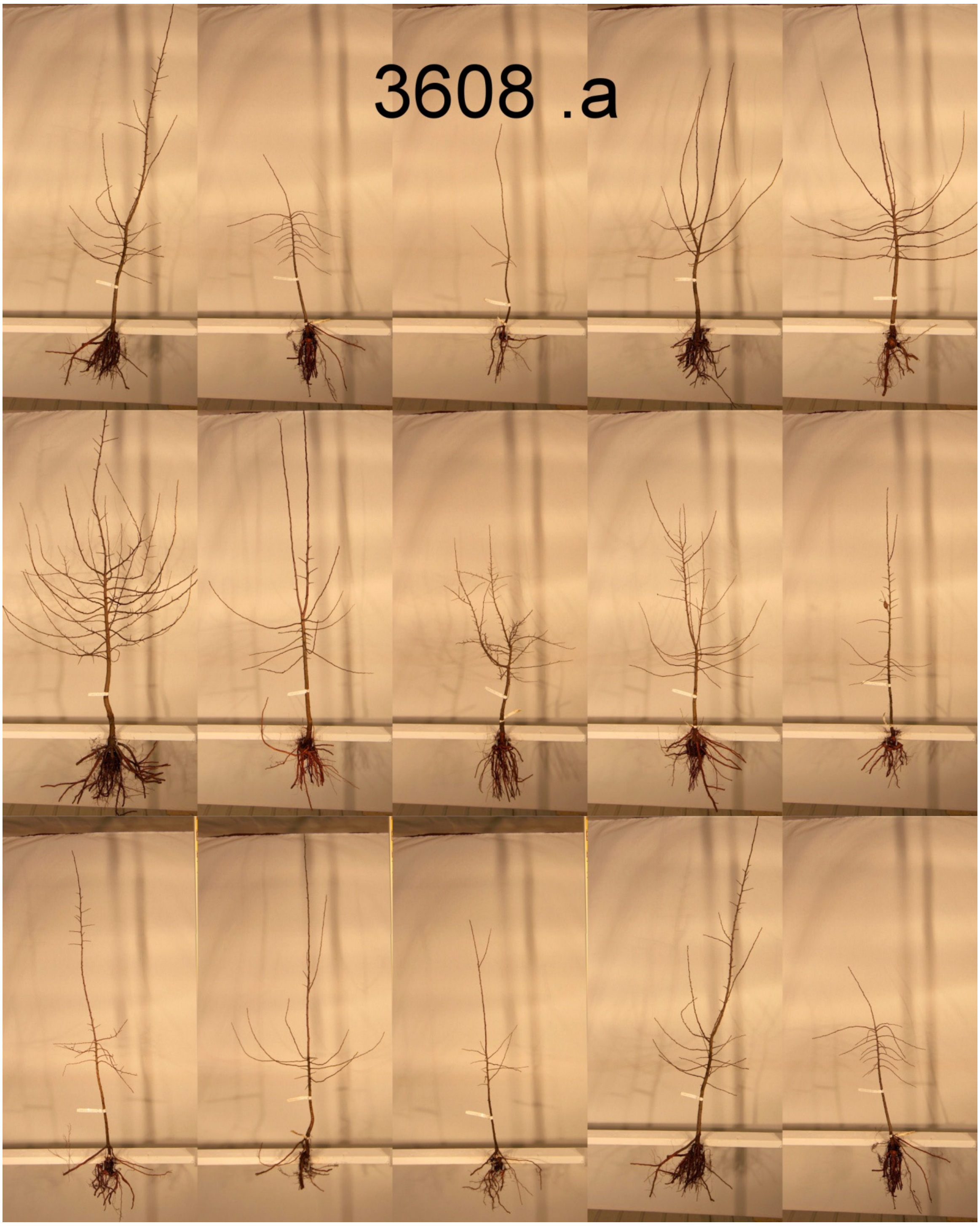

Fig. 2. Sampling of high resolution images of seedlings derived from the GMAL 3608.a mother tree and several pollen pools. What is striking about this group of half sib trees is the more flat branching that may be correlated with productive fruiting wood.

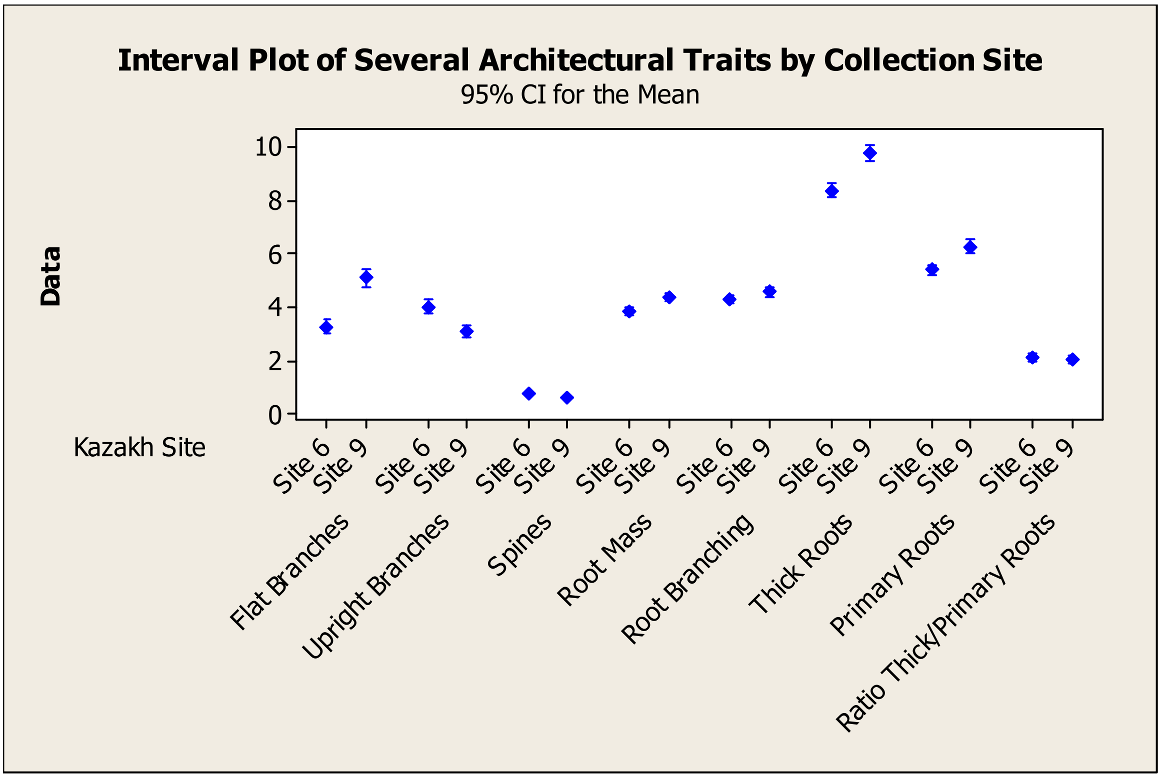

Fig. 3. Comparison of means for several apple seedling architectural traits grouped by collection sites in Kazakhstan. Trees from Site 9 seem to possess more flat branches, have higher root mass and thicker primary roots. Compared to Site 6, Site 9 was at higher elevation and had less precipitation.