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Full Length Article

Rootstocks Influence Morphological and Biochemical Changes in Young 'Red Fuji' Apple Plants

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Abstract

Dwarfing rootstocks are important germplasm resource for high density plantings to get maximum production potential. This study was conducted to examine the effects of different rootstocks on growth morphology, hormones, minerals and carbohydrate concentrations among different scion/rootstock combinations of an apple cultivar 'Red Fuji'. The results indicated that 'Red Fuji'/'M-9' had lower growth vigour based on their lower node number, shorter internode length and primary shoot length and reduced trunk diameter of scion. Nutrient analysis showed that 'Red Fuji'/'M-9' had a lower mineral elements concentration such as phosphorous (P), potassium (K), magnesium (Mg), zinc (Zn) and copper (Cu) in the leaf compared with 'Red Fuji' apple grafted onto 'M-26', 'Chistock-1' and 'Baleng' rootstocks that likely reduced the growth rate. Further, we observed that 'Red Fuji'/'M-9' had a lower concentration of indole-3-acetic acid (IAA), gibberellic acid (GA_3) and zeatin riboside (ZR) and higher abscisic acid (ABA) concentration in the roots compared with 'Baleng', 'Chistock-1' and 'M-26'. The starch content was also higher in the leaf and root of 'Red Fuji' apple grafted onto 'M-9' rootstock compared with more vigorous rootstocks. Preliminary correlation analysis and factor analysis showed that primary shoot length, plant height and scion trunk diameter (tree morphology index), starch content in the leaf, indole-3-acetic acid, zeatin riboside, gibberellic acid, abscisic acid, P, calcium (Ca) and iron (Fe) contents in the roots can be considered as main morphological and bio-chemical parameters influenced by different rootstocks. This study concluded that morphological parameters (lower plant height), lower hormonal ratio ($IAA+GA_3+ZR$)/ABA), lower mineral (P, K and Mg) and higher starch content could be used as indices for selection of dwarfing apple rootstocks. © 2019 Friends Science Publishers

Keywords: Apple; Dwarfing mechanism; Endogenous hormones; Grafting; Ion uptake; Rootstock

Introduction

Dwarfing apple (*Malus domestica* Borkh.) rootstocks are getting popularity in apple producing areas across the world because they reduces the vegetative growth of scion cultivars, increase yield and reduce labour costs for production practices such as spraying, pruning and harvesting (Robinson *et al*., 2011). The physiological mechanism underlying rootstock-induced vigour has been debated by many authors in the past 70 years (Webster, 1995). Several studies have been conducted to verify the rootstock-induced dwarfing effect, but the related mechanisms have not been clarified. Scion vigour is known to be influenced by numerous factors including mineral transport (Fallahi *et al*., 2002), hormone levels (Van Hooijdonk *et al*., 2011), phenolic contents (Yildirim *et al*., 2016) and carbohydrates accumulation (Foster *et al*., 2017).

The studies about the physiological and biochemical mechanisms of dwarfing apple rootstocks have mainly focused on water and mineral transport, hormone levels, anatomical structures and photosynthetic characteristics (Han *et al*., 2013). Rootstocks influenced the scion mineral composition in apple trees and the differences between rootstock-scion interactions were associated with mineral uptake capacity of rootstocks due to their changing root structure (Kviklys *et al*., 2017). Al-Hinai and Roper (2004) further reported that rootstock-scion interactions resulted differences in absorption and transport of mineral nutrients to the scion. The relation between the reduction of scion vigour in dwarfing apple plants and the altered shoot–root–shoot chemical signaling has been reported, such hormones as auxin, cytokinin, gibberellins and abscisic

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acid (Kamboj *et al*., 1999; Van Hooijdonk *et al*., 2011). Lower concentrations of growth promoting hormones (IAA, Ck and GA) and higher concentration of growth inhibiting hormone (ABA) in dwarfing rootstocks has been studied repeatedly in grafted plants, but clear conclusion for the hormonal dependence of scion vigour is still lacking (Gregory *et al*., 2013).

Size-controlling rootstocks could affect the morphological parameters of grafted apple cultivars differentially, which includes shoot length, internodal length, branch composition, trunk cross-sectional area (TCSA) and yield (Van Hooijdonk *et al*., 2011; Karlidag *et al*., 2014). Therefore, the selection of dwarfing rootstocks and screening for superior rootstocks from apple hybrid seedlings are necessary for improving the cultivation methods used for apple production (Zhao *et al*., 2016).

The high-density apple cultivation favored by the utilization of dwarfing rootstocks could greatly promote the apple yield. Current research was mainly focused on analyzing the changes in endogenous hormones, mineral nutrients, carbohydrates levels and scion growth of 'Red Fuji' apple grafted onto different rootstocks. We wanted to investigate how rootstocks induce changes in scion growth by altering the uptake of mineral nutrients and hormones and carbohydrate levels at early stages of growth development. In this study, we investigated the mechanism of dwarfing and the possible influences on 'Red Fuji' apple grafted onto 'Baleng', 'Chistock-1', 'M-26' and 'M-9' rootstocks.

Materials and Methods

Plant Materials and Cultivation Conditions

The experiment was conducted at Apple Research Orchard of China Agricultural University, Beijing (Latitude 39°54'N longitude 116°24'E). During March 2017, 'Red Fuji' scions were collected from 7-years old trees and cleft-grafted at a height of 35 cm onto 1-year-old rootstocks of 'Baleng' (vigorous), 'Chistock-1' (semi-dwarf), 'M-26' (semi-dwarf) and 'M-9' (dwarf) (Table 1). Trees were planted in 30 cm diameter plastic pots containing a mixture of garden soil, nursery substrate and sand (3:2:1) and grown in a green house. The plants were drip irrigated but no fertilizer was applied during the study period. The experiment was carried out in a randomized complete block design (RCBD) with four biological replications (each replicate contain two plants), and in total 192 plants were used in whole study.

Measurement of Plant Growth

From May 2017 to October 2017, plant height (cm), primary shoot length (cm), node number and average internodal length (cm) were measured for the scion. Trunk diameter was measured at three points including scion stem (5 cm above the graft junction), graft junction and rootstock stem (5 cm below the graft junction) by using digital Vernier caliper. The data of these traits were measured at 60, 90, 120, 150, 180 and 210 days after grafting (DAG) for each grafting combination. For the measurement of root–shoot ratio, healthy plants at the same growth stage were removed from the pots and washed with clean water before the leaves dropped in the autumn of 2017.

Mineral Analysis

To measure the mineral concentration of leaves and roots, tissues were sampled at 60, 90, 120, 150, 180 and 210 DAG. The dried samples were ground using a Cyclotec Sample Mill (Hoganas, Sweden) and passed through a 40 mesh screen. Then the powder was digested in a mixture of H_2SO_4–H_2O_2. The nitrogen (N) concentration was determined according to Kjeldahl method (Nelson and Sommers, 1980). The concentrations of other mineral elements (P, K, Ca, Mg, Mn, Fe, Zn and Cu) were determined using ICP-MS according to the methods described by Masson *et al.* (2010). The results were expressed on dry matter basis: g kg^{-1} for macro elements (N, P, K, Ca and Mg) and mg kg^{-1} for microelements (Mn, Fe, Cu and Zn).

Endogenous Hormones Analysis

The samples for hormones were collected from different scion-rootstock combinations at 60, 90, 120, 150, 180 and 210 DAG. The endogenous hormones containing (IAA, ZR, GA_3 and ABA contents) were extracted from the frozen leaf, scion-bark, rootstock-bark and root samples using a method of enzyme linked immunosorbent assays (ELISA) technique according to the procedure described by Zhao *et al*. (2006). The quantification of hormones was calculated based on a standard curves and expressed as ng g^{-1} fresh weight.

Starch and Soluble Carbohydrates Analysis

The samples for starch and soluble carbohydrates measurements were collected at 60, 90, 120, 150, 180 and 210 days after grafting (DAG). Leaf and root soluble carbohydrate contents were extracted by high-performance liquid chromatography (HPLC) using a method described by Filip *et al.* (2016). The starch concentrations were analyzed according to the procedure described by Changjie *et al.* (1998). The amount of soluble carbohydrates and starch were reported as mg g^{-1} DW.

Statistical Analysis

Experimental data were analyzed using statistical package SPSS 19.0 (SPSS Inc, Chicago, USA) for correlation analysis and analysis of variance (ANOVA) to perform the statistical analysis. Mean comparisons among the treatments were evaluated by least significant difference (LSD) multiple comparison tests at $P< 0.05$.

Results

Plant Growth Measurement

Apple rootstocks influenced the morphological parameters of grafted apple trees. 'Red Fuji' apple grafted onto 'Baleng' rootstock had the longest primary shoot length and vigorous growth. The weakest growth vigour and the shortest primary shoot length (96.6 cm) were obtained for 'Red Fuji' grafted onto 'M-9' rootstock. Seven months after grafting, significant differences were observed in the growth parameters of the 'Red Fuji' apple grafted onto the different rootstocks (Table 2). The numbers of nodes (40.167) were more for plants grafted onto 'Baleng' rootstock compared with 'Chistock-1', 'M-26' and 'M-9' rootstocks. Trees grafted onto 'Baleng' and 'Chistock-1' rootstocks produced longer internodal length (3.10 cm), whereas the 'M-9' rootstock resulted in the smallest internodal length (2.13 cm). Trunk diameter of 'Red Fuji' apple (8.10 mm) was significantly greater when it was grafted onto the 'Baleng' and 'Chistock-1' rootstocks compared with other rootstocks. Among the four scion/rootstock combinations higher plant growth was induced by 'Baleng' rootstock whereas, 'M-9' rootstock induced dwarfing.

Mineral Nutrient Concentrations

In this study, rootstock behaved differentially regarding mineral uptake and accumulation in their leaves and roots when 'Red Fuji' scion was grafted onto different rootstocks (Fig. 2). Leaf N concentrations were higher at early stages and subsequently decreased with the progressing season. Red Fuji grafted on 'M-9' rootstock had higher leaf N concentrations (26.16 g kg^{-1}) compared with other rootstocks. The lower P concentrations (by 1.774 and 1.093 g kg^{-1}, respectively) were recorded in the leaves and roots of 'M-9' rootstock compared with semi-dwarf ('M-26' and 'Chistock-1') and vigorous rootstock ('Baleng'). Red Fuji grafted onto 'Baleng' rootstock had significantly higher K concentrations (11.08 g kg^{-1}) in their leaves compared with other rootstocks. The higher Mg concentrations (by 6.944 and 3.18 g kg^{-1}, respectively) were recorded in the leaves and roots of 'Baleng' rootstock compared with 'M-9', 'M-26' and 'Chistock-1' rootstocks. The higher concentrations of microelements (Ca and Mn) in leaves (by 12.39 g kg^{-1} and 60.44 mg g^{-1}) and roots (by 8.67 g kg^{-1} and 36.81 mg g^{-1}) were recorded in the 'M-9' rootstock compared with 'M-26', 'Chistock-1' and 'Baleng'. The concentrations of (Cu and Zn) remained at lowest level (by 4.663 and 4.699 mg g^{-1}) and (24.20 and 15.24 mg g^{-1}) in the leaves and roots of 'M-9' rootstock while the other three rootstocks accumulated higher concentrations. The higher concentrations of Fe (144.75 mg g^{-1}) were observed in the leaves of 'Red Fuji' grafted onto 'M-9' rootstock and lower concentration was observed for 'Red Fuji' grafted onto 'M-26', 'Chistock-1' and 'Baleng' rootstocks, respectively.

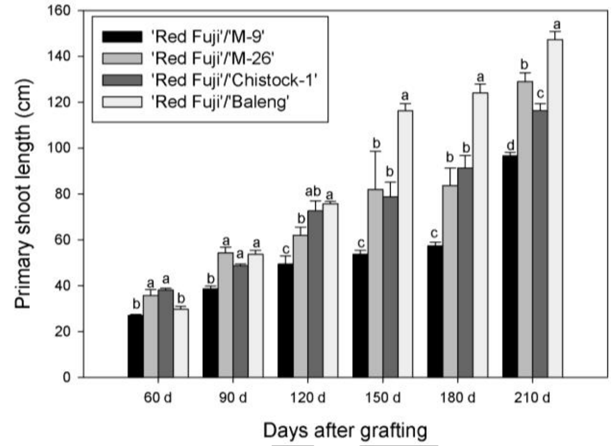

Fig. 1: Changes of primary shoot length in 'Red Fuji' apple cultivar grafted onto 'M-9', 'M-26', 'Chistock-1' and 'Baleng' rootstocks at different stages of growth and development. Error bars show the standard errors of five biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

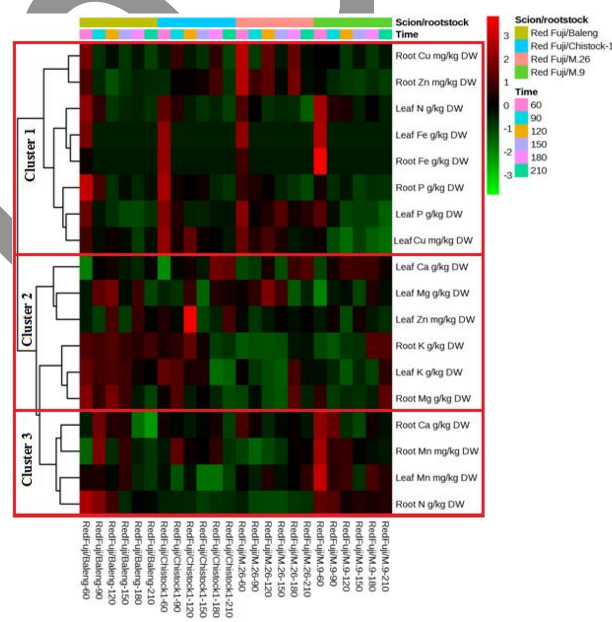

Fig. 2: Hierarchical cluster analysis of leaf and root mineral nutrient concentrations. The color scale from high (red) to low (green) at the top of the figure indicates the log-transformed values of the mineral nutrient concentrations in 'Red Fuji'/'M-9', 'Red Fuji'/'M-26', 'Red Fuji'/'Chistock-1' and Red Fuji/'Baleng' samples taken at 60, 90, 120, 150, 180 and 210 days after grafting

Hierarchical Cluster Analysis for Mineral Elements

Hierarchical cluster analysis was applied to make a cluster for minerals. The accumulation patterns of different mineral elements regarding different scion-rootstock combinations and sampling time are presented in Fig. 2. During sampling stages, minerals showed a similar pattern and were grouped into three main clusters according to the calculated distance.

Table 1: The vigour, description and origin of different rootstocks used in this study

Rootstock	Parentage	Origin	Vigour	Reference
'M-9'	unknown	HRI-East Malling, UK	Dwarf	Webster and Wertheim (2003)
'M-26'	*Malling 26* ('M.16' x 'M-9')	HRI-East Malling, UK	Semi-dwarf	Webster and Wertheim (2003)
'Chistock-1'	*Malus xiaojinensis*	China	Semi-dwarf	Han *et al.* (2013)
'Baleng'	*Malus robusta*	China	Vigorous	An *et al.* (2017)

Table 2: Effect of different rootstocks on the growth parameters of 'Red Fuji' apple

Rootstocks	No. of nodes	Internodal length (cm)	Plant height (cm)	Trunk diameter of scion (mm)	Trunk diameter of rootstock (mm)	Trunk diameter of graft union (mm)
'M-9'	43.7 b	2.133 b	112.2 c	4.70 c	12.20 c	14.93 b
'M-26'	44.6 b	2.666 ab	135.3 b	6.83 b	17.03 b	16.73 ab
'Chistok-1'	53.0 a	2.833 ab	150.6 a	7.76 ab	19.20 a	18.63 a
'Baleng'	57.0 a	3.100 a	159.3 a	8.10 a	16.73 b	17.46 ab

The data are means of five biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

Regarding passage of time (sampling time), the decreasing and increasing trends of mineral element concentrations were showed in cluster 1 and cluster 2, respectively. In cluster 3, the irregular pattern was observed at different sampling times.

Endogenous Hormone Levels

Different rootstocks significantly affected the endogenous hormonal levels in the grafted 'Red Fuji' apple (Fig. 3 and 4). The leaf IAA level (60.89 ng g^{-1}) was higher for 'Red Fuji' grafted onto 'M-26' and 'Baleng' rootstock whereas leaf IAA levels (45.65 ng g^{-1}) were lower for plants grafted onto 'M-9' rootstock (Fig. 3A). The IAA content (51.17 ng g^{-1}) in the scion bark was highest with 'Baleng' rootstock and lower (43.26 ng g^{-1}) in the dwarf ('M-9') and semi-dwarf rootstocks ('Chistock-1' and 'M-26') in Fig. 3B. Similar IAA contents were detected in the rootstock bark, being lowest (39.102 ng g^{-1}) for 'M-9' rootstock and higher (by 43.50, 43.70 and 44.71 ng g^{-1}, respectively) contents were observed for the 'Baleng', 'M-26' and 'Chistock-1' rootstocks (Fig. 3C). Root IAA levels (32.02 ng g^{-1}) were significantly higher for 'Baleng' rootstock compared with other rootstocks (Fig. 3D). The leaf ZR levels (13.85 ng g^{-1}) were apparently higher in the 'Red Fuji' grafted onto 'Baleng' rootstock compared with 'Red Fuji' plants grafted onto 'M-9', 'M-26' and 'Chistock-1' rootstocks (Fig. 3E). The ZR levels (6.691 ng g^{-1}) in the scion bark showed lowest with 'M-9' rootstock whereas other rootstocks produced higher IAA contents (Fig. 3F). Similar ZR levels (10.83 ng g^{-1}) were detected in the rootstock bark, being lowest in 'M-9' rootstock and higher levels (by 13.98, 20.37 and 21.08 ng g^{-1}, respectively) were observed for 'M-26', 'Chistock-1' and 'Baleng' rootstocks (Fig. 3G). Root ZR levels (4.610 ng g^{-1}) were significantly lowest for 'M-9' rootstock compared with other rootstocks (Fig. 3H).

The leaf GA_3 levels (8.348 ng g^{-1}) were markedly higher for 'Red Fuji' grafted onto 'Baleng' rootstock, whereas leaf GA_3 levels (by 6.603, 6.796 and 7.698 ng g^{-1}, respectively) were lower for plants grafted onto 'M-9', 'M-26' and 'Chistock-1' rootstocks (Fig. 4A). The

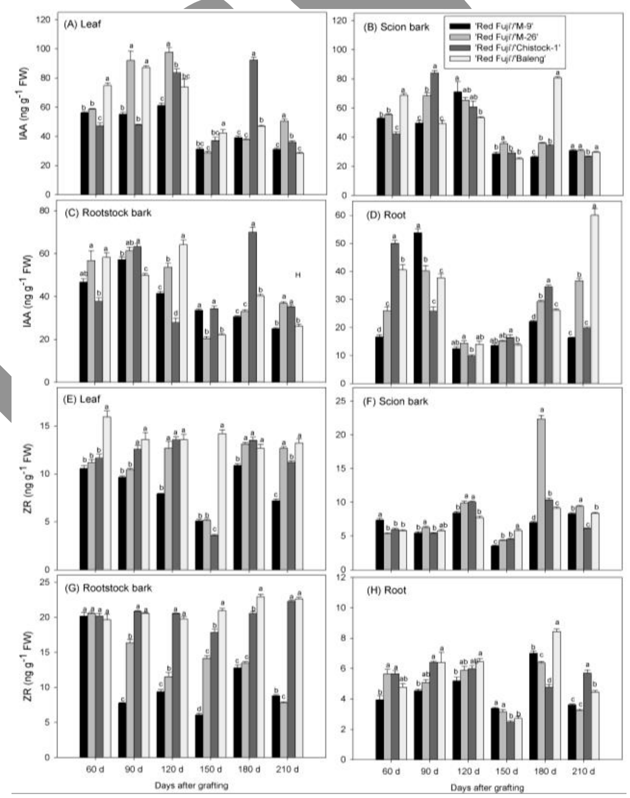

Fig. 3: Endogenous hormone levels in different tissue of 'Red Fuji' apple grafted onto different rootstocks at different sampling points (x-axis). Endogenous indole-3-acetic acid (IAA) levels of leaf (A), scion-bark (B), rootstock-bark (C), root (D); endogenous zeatin riboside (ZR) levels of leaf (E), scion-bark (F), rootstock-bark (G), and root (H). Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

GA_3 levels (6.644 ng g^{-1}) in the scion bark were also highest with the 'Baleng' rootstock than other rootstocks (Fig. 4B). Similar GA_3 levels were detected in the rootstock bark, being lowest in 'M-9' rootstock and higher levels were observed for 'Red Fuji' grafted onto 'M-26', 'Chistock-1' and 'Baleng' rootstocks (Fig. 4C).

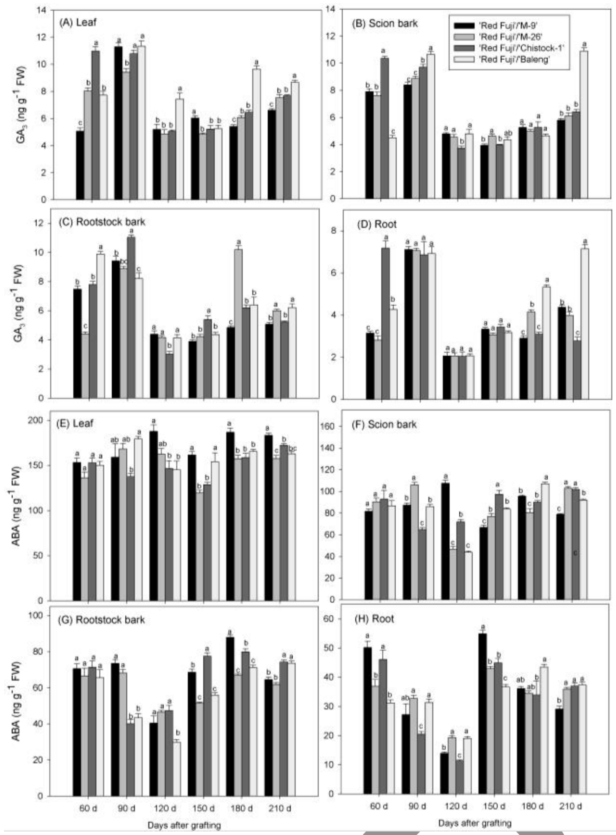

Fig. 4: Endogenous hormone levels in different tissue of 'Red Fuji' apple grafted onto different rootstocks at different sampling points (x-axis). Endogenous gibberellic acid (GA_3) levels of leaf (A), scion-bark (B), rootstock-bark (C), root (D); endogenous abscisic acid (ABA) levels of leaf (E), scion-bark (F), rootstock-bark (G) and root (H). Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

Root GA_3 levels (3.792 and 3.856 ng g^{-1}, respectively) were lowest for 'M-9' and 'M-26' rootstocks compared with Chistock-1 and Baleng rootstocks (Fig. 4D). The leaf ABA levels (174.6 ng g^{-1}) were significantly higher for 'Red Fuji' grafted onto 'M-9' rootstock, whereas leaf ABA levels (149.5, 150.2 and 159.4 ng g^{-1}, respectively) were lower for plants grafted onto 'Chistock-1', 'M-26' and 'Baleng' rootstocks (Fig. 4E). Similar ABA levels (67.15 ng g^{-1}) were detected in the rootstock bark being highest in 'M-9' rootstock and lower levels were observed for the 'M-26', 'Chistock-1' and 'Baleng' rootstocks (Fig. 4G). Root ABA contents (34.54 ng g^{-1}) were higher for 'M-9' rootstock whereas 'M-26', 'Chistock-1' and 'Baleng' rootstocks produced less ABA in the root tissues (Fig. 4H). The combine ratio (0.564 ng g^{-1}) of leaf, scion bark, rootstock bark and root ($IAA+ZR+GA_3$)/ABA were significantly lower for 'M-9' rootstock whereas higher (by 0.743, 0.756 and 0.802 ng g^{-1} respectively) ratio was observed for 'M-26', 'Chistock-1' and 'Baleng' rootstocks (Fig. 5).

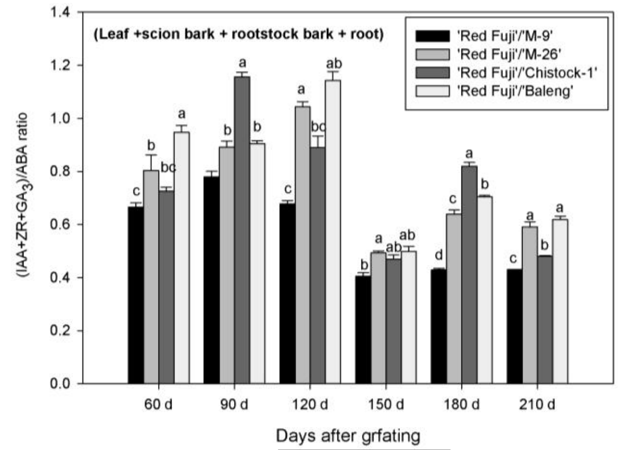

Fig. 5: Endogenous ($IAA+ZR+GA_3$)/ABA ratio in different tissues of 'Red Fuji' apple grafted onto rootstocks at different sampling points (x-axis). Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$). IAA = indole-3-acetic acid. ZR = zeatin riboside. GA_3 = gibberellic acid. ABA = abscisic acid

Starch and Total Carbohydrate Content

Different rootstocks significantly affected the starch and total measured carbohydrate content in the grafted 'Red Fuji' apple cultivar grafted onto different rootstocks (Table 3). Starch content in leaves and roots were obviously influenced by rootstocks and scion combinations. 'Red Fuji' apple grafted on 'M-9' rootstock exhibited higher concentrations (4.556 mg g^{-1}) of starch in their leaves, whereas lower starch content (by 4.185, 4.278 and 4.325, respectively) was observed for 'Chistock-1', 'Baleng' and 'M-26' rootstocks. Root starch content (21.95 mg g^{-1}) was significantly higher with 'M-9' rootstock and decreased (18.03, 16.86 and 9.35 mg g^{-1} respectively) when grafted onto 'M-26', 'Baleng' and 'Chistock-1' rootstocks. Hence the results showed that the starch increased/or decreased according to the decreasing or increasing level of vigour capacity of the rootstocks. Total carbohydrate content in the roots was found to be similar to the starch content influenced by the rootstock and the scion. Trees on 'Chistock-1' and 'M-26' rootstocks had significantly (higher by 42.29 and 37.23 mg g^{-1} respectively) total carbohydrate content in their leaves, while the other rootstocks treatment had lower carbohydrate content. In case of root, the 'M-9' had the maximum carbohydrate contents (40.80 mg g^{-1}) while the minimum carbohydrates (31.60 mg g^{-1}) were recorded for 'Chistock-1' rootstock.

Correlation Analysis for the Morphological and Biochemical Indices of 'Red Fuji' Apple with Different Rootstocks

Some statistically significant correlations were found among morphological and bio-chemical indices related to plant vigour (Table 4). Number of nodes, shoot length and plant

Table 3: Changes in carbohydrate and starch content in the leaves and roots of ‘Red Fuji’ apple grafted onto different rootstocks measured at 60, 90, 120, 150, 180 and 210 days after grafting (DAG)

Parameters	Rootstocks	Plant tissues	60 DAG	90 DAG	120 DAG	150 DAG	180 DAG	210 DAG	Average
Starch (mg/g^{-1} FW)	‘M-9’	Leaf	4.336 b	4.430 b	4.436 c	3.756 b	3.740 b	6.636 a	4.556 a
		Root	7.893 b	8.040 b	16.70 b	18.22 c	32.38 b	48.46 a	21.95 a
	‘M-26’	Leaf	4.743 a	4.850 a	5.190 a	3.766 b	3.376 c	4.053 c	4.325 b
		Root	8.390 a	8.516 a	22.97 a	23.15 a	16.21 d	28.94 b	18.03 b
	‘Chistock-1’	Leaf	3.706 c	3.823 c	4.716 b	4.430 a	4.636 a	3.796 d	4.185 c
		Root	3.246 d	3.343 d	12.86 c	3.796 d	19.66 c	13.23 d	9.35 d
	‘Baleng’	Leaf	4.246 b	4.450 b	3.193 d	4.523 a	4.533 a	4.726 b	4.278 b
		Root	6.906 c	7.010 c	8.41 d	20.25 b	34.82 c	23.80 c	16.86 c
Total measured carbohydrates (mg/g^{-1} FW)	‘M-9’	Leaf	33.11 b	34.72 b	25.19 bc	12.33 b	36.24 ab	40.63 b	30.32 c
		Root	23.38 a	32.1 ab	40.80 a	35.19 c	53.52 b	59.85 a	40.80 a
	‘M-26’	Leaf	39.33 a	49.81 a	28.74 b	27.15 a	36.14 ab	42.18 b	37.23 b
		Root	18.95 b	33.66 a	43.38 a	43.43 b	37.57 c	53.83 b	38.30 b
	‘Chistock-1’	Leaf	36.54 ab	50.94 a	38.25 a	19.01 a	45.83 a	48.14 a	41.45 a
		Root	17.48 b	16.23 c	23.11 c	20.67 d	34.05 c	28.17 d	23.29 c
	‘Baleng’	Leaf	22.92 c	51.01 a	21.24 c	22.97 a	30.83 b	40.63 b	31.60 c
		Root	15.97 b	29.56 b	33.19 b	50.28 a	62.77 a	43.87 c	39.27 b

Different letters indicate significant differences by LSD ($P \leq 0.05$). DAG = days after grafting

Table 4: Correlations of the morphological and biochemical indices of ‘Red Fuji’ apple trees with the different rootstocks

Parameters	Number of nodes	Internodal length	Shoot length	Plant height	Scion trunk diameter	Rootstock trunk diameter
Number of nodes	-	0.652	0.964	0.931	0.949	0.277
Internodal length	0.652	-	0.809	0.868	0.842	0.288
Shoot length	0.964	0.809	-	0.994	0.971	0.417
Plant height	0.931	0.868	0.994	-	0.970	0.424
Scion trunk diameter	0.949	0.842	0.971	0.970	-	0.197
Rootstock trunk diameter	0.277	0.288	0.417	0.424	0.197	-
IAA (leaf)	0.626	0.881	0.809	0.855	0.725	0.707
IAA(scion bark)	0.717	0.991	0.842	0.892	0.894	0.203
IAA (rootstock bark)	0.738	0.691	0.852	0.859	0.712	0.828
IAA (root)	0.831	0.929	0.895	0.921	0.964	0.092
ZR (leaf)	0.891	0.900	0.937	0.953	0.989	0.143
ZR (rootstock bark)	0.991	0.600	0.955	0.917	0.909	0.382
ZR (root)	0.987	0.757	0.980	0.964	0.987	0.243
GA^3 (leaf)	0.984	0.616	0.919	0.882	0.944	0.102
GA^3 (scion bark)	0.975	0.481	0.902	0.848	0.855	0.325
GA^3 (rootstock bark)	0.849	0.672	0.917	0.907	0.795	0.736
GA^3 (root)	0.920	0.693	0.870	0.852	0.951	-0.082
ABA (leaf)	-0.509	-0.587	-0.668	-0.688	-0.492	-0.942
ABA (scion bark)	-0.662	-1.000	-0.813	-0.870	-0.851	-0.267
ABA (rootstock bark)	-0.613	-0.973	-0.743	-0.802	-0.830	-0.060
ABA (root)	-0.933	-0.723	-0.975	-0.961	-0.892	-0.591
Starch leaf	-0.758	-0.651	-0.855	-0.853	-0.710	-0.827
Starch root	-0.678	-0.201	-0.659	-0.604	-0.475	-0.772
Total carbohydrates (leaf)	0.277	0.091	0.356	0.336	0.120	0.966
Total carbohydrates (root)	-0.433	0.122	-0.380	-0.310	-0.172	-0.734
N (Leaf)	-0.292	-0.640	-0.514	-0.569	-0.366	-0.878
N (Root)	0.042	-0.153	-0.135	-0.163	0.080	-0.944
P (Leaf)	-0.098	0.364	0.141	0.205	-0.020	0.832
P (Root)	0.963	0.667	0.973	0.947	0.899	0.526
K (Leaf)	0.935	0.667	0.876	0.853	0.949	-0.060
K (Root	0.805	0.310	0.645	0.587	0.740	-0.312
Ca (Leaf	-0.679	-0.983	-0.802	-0.856	-0.874	-0.123
Ca (Root)	-0.971	-0.742	-0.993	-0.977	-0.942	-0.470
Mg (Leaf)	0.533	0.983	0.727	0.799	0.739	0.378
Mg (Root)	0.703	0.388	0.569	0.535	0.720	-0.489
Fe (Leaf)	-0.848	-0.955	-0.945	-0.974	-0.958	-0.328
Fe (Root)	-0.673	-0.999	-0.828	-0.884	-0.852	-0.324
Mn (Leaf)	-0.711	-0.316	-0.722	-0.679	-0.541	-0.811
Mn (Root)	-0.194	-0.812	-0.448	-0.538	-0.407	-0.542
Zn (leaf)	0.627	0.011	0.550	0.475	0.376	0.646
Zn (Root)	-0.114	0.424	0.138	0.212	0.001	0.769
Ratio (IAA+GA_3+ZR/ABA)	0.963	0.667	0.973	0.947	0.899	0.526

IAA = indole-3-acetic acid. ZR = zeatin riboside. GA_3 = gibberellic acid. ABA = abscisic acid. P = phosphorus. N = ntirgen. K = potash. Ca = calcium. Mg = magnesium. Fe = iron. Mn = manganese. Zn = Zinc

height were also positively correlated with IAA content of leaves, scion bark, rootstock bark and roots. Plant height and trunk diameter of scion were significantly positively correlated with the roots of ZR contents. Number of nodes was significantly positively correlated with leaves and scion bark of GA_3 content. Plant height and shoot length were significantly negatively correlated with ABA content of roots. In case of nutrient, the content of nitrogen, calcium, iron and manganese in the leaves and roots were negatively correlated with Internodal length, shoot length and plant height. The starch content of leaves and roots were negatively correlated with the number of nodes, internodal length, shoot length, plant height and trunk diameter of both scion and rootstock. In comparison, these morphological parameters were positively correlated with the ratio of (IAA+GA_3+ZR)/ABA.

Indices Linked to the Dwarfing Effects of Dwarf Rootstocks

Factor analysis showed that the eigenvalues (*Ei*) of the first three components were greater than 1, representing the main factors. Eigen values are the variances of the principal components because we conducted our principal component analysis of the correlation matrix, the variables are standardized, which means that each variable has a variance of 1 and the total variance is equal to the number of variables used in the analysis. The sum of the percent of variance (POV) was 100%, with the amount of information contained in these three factors representing 100% of the total information (Table 4). Component 1 showed highest (60.29%) of total variability with the greatest loading of shoot length (0.991). Representative indices of component 2 were trunk diameter of rootstock, ZR (scion bark), N (leaf, root), P (leaf), K, Mg and Zn (root), with POVs of 23.917%. Component 3 had POV of 15.793% with the representative index for Zn in the leaves, magnesium, starch and total measured carbohydrates in the roots. Furthermore, results showed that plant height, shoot length, number of nodes, trunk diameter of scion (tree morphology index), hormonal contents *i.e.*, IAA content in the (leaves and rootstock bark), ZR content in the (leaves, rootstock bark and roots), GA_3 content in the rootstock bark, ABA content in the roots and ratio (IAA+GA_3+ZR/ABA) are the main morphological and hormonal indices that plays important role in dwarfism. In case of nutrients P, Fe and starch in the leaves; N, P, Ca, Mn and Fe content in the roots are suitable indices for evaluating the dwarfing characters of different size controlling rootstock.

Discussion

Rootstocks significantly affect the growth traits of grafted apple trees. Marini *et al*. (2009) observed that the tree vigour and canopy size of 'Golden Delicious' apple grafted onto P.22 rootstock was significantly larger compared with 'Golden Delicious' plants grafted onto 'M-9' rootstock. There were marked differences in the tree vigour and canopy size of the Clementine peach cultivar grafted onto different rootstocks; both parameters were 10% smaller on dwarf rootstock compared with vigorous rootstock (Bassal, 2009). In the present study, the 'Red Fuji' apple grafted onto more vigorous rootstocks such as 'Baleng', 'Chistock-1', and 'M-26', rootstock increased shoot length and consequently the tree size of young apple trees was increased showing vigorous characteristics. Various studies have suggested that apple scions grafted onto vigorous rootstocks have longer shoot lengths, greater TCSA and stronger growth vigour (Gjamovski and Kiprijanovski, 2011; Amiri *et al*., 2014). In contrast, the 'Red Fuji' apple grafted onto 'M-9' rootstock has shorter shoot length, and a reduced growth, showing dwarfing characteristics.

Differences in the mineral concentrations of grafted trees can be explained by the genetic effect leading to different nutrient uptake ability of rootstocks (Kucukyumuk and Erdal, 2011). In the present study, 'Red Fuji' apple grafted onto 'M-9' rootstock accumulated higher concentrations of N, Ca, Mn and Fe in their leaves compared with 'Red Fuji' apple grafted onto 'M-26', 'Chistock-1' and 'Baleng' rootstocks. Various studies have suggested that trees grafted onto dwarf rootstocks accumulated higher N concentrations as compared with trees grafted onto more vigorous rootstocks (Aguirre *et al*., 2001; Amiri *et al*., 2014). On the other hand, 'M-9' rootstock had lower mineral consumption by the least vegetative growth such as plant height, shoot length, number of nodes, and trunk diameter of scion (Fig. 1 and Table 2), which resulted in higher accumulation of minerals in the leaves (Amiri *et al*., 2014). Among rootstocks it was clearly seen that 'Red Fuji' apple grafted onto 'M-9' rootstock had significantly lower (P, K) mineral nutrient concentrations, compared with 'Red Fuji' apple grafted onto 'M-26', 'Chistock-1' and 'Baleng' rootstocks. The higher Ca and lower Mg content were exhibited on 'M-9' dwarfing rootstock compared with vigorous rootstocks (Fallahi and Mohan, 2000) which is in agreement with the results of our study. In the present study 'Red Fuji' apple grafted onto 'M-9' rootstock was proved as less efficient for the absorption of some nutrients (P, K Mg and Zn) from the medium. It can be concluded that the reduction of uptake capacity was associated in apple dwarfing rootstocks to their smaller root system (Kucukyumuk and Erdal, 2011) and each rootstock exhibits a range of size-controlling potential and may have a different potential of transport rate of raw sap (amount of minerals) from root to leaf (Tombesi *et al*., 2011; Tworkoski and Fazio, 2016).

Previous studies have indicated that tree growth vigour is restricted by hormone synthesis and transport (Van Hooijdonk *et al*., 2011). Cytokinins and auxin enhance tree growth and the sprouting of axillary buds. Gibberellic acids (GA) favour internodal elongation, while ABA promotes tree ageing (Yu *et al*., 2012). In the present study, the IAA levels were positively correlated with the growth vigour of the grafted plants. Additionally, 'Red Fuji' apple grafted onto 'M-9' rootstock exhibited lower IAA levels in their roots compared with 'M-26', 'Chistock-1' and 'Baleng' rootstocks. Various studies suggest that the trees grafted onto dwarf rootstocks received a less amount of IAA in their roots compared with trees grafted onto semi-dwarf and vigorous rootstocks (Kamboj *et al*., 1999; Michalczuk, 2002). The expression of the polar auxin related gene *(PIN-1)* was distinctly reduced in shoots of apple grafted onto a dwarf rootstock; the change in gene expression and reduced basipetal auxin translocations resulted in an inadequate supply of IAA to the roots of the apple (Liu *et al*., 2017). This action inhibits internode elongation and axillary bud sprouting that shortens internodal length, weakens growth vigour and leads towards dwarfing (Aloni *et al*., 2010; Li *et al*., 2012).

This study showed that, 'Red Fuji' apple grafted onto 'M-9' rootstock had a lower amount of cytokinin (ZR) and gibberellins (GA_3) in their leaf and root compared with 'Red Fuji' apple grafted onto 'M-26', 'Chistock-1' and 'Baleng' rootstocks (Fig. 3 and 4). Leaf, scion bark and root GA levels of the grafted citrus plants were also lowest on the Red tangerine and highest on the Canton lemon rootstock (Liu *et al*., 2017). The reduction of scion growth in 'Red Fuji' apple grafted onto 'M-9' rootstock indicated a reduced IAA transport from scion to root, which in turn also decreased the consequent amount of root-derived cytokinins and gibberellins transported to the scion causing a dwarf tree phenotype (Van Hooijdonk *et al.*, 2010). The higher concentration of ABA may have increased phloem differentiation and the resulting high bark to wood ratio could have reduced xylem development in more dwarfing rootstocks (Tworkoski and Miller, 2007). It can be concluded that the differences in ABA levels could be responsible for differences in growth vigour of grafted plants.

In the present study, starch levels were negatively correlated with the growth vigour of grafted apple trees. Additionally, 'Red Fuji' apple grafted onto 'M-9' rootstock accumulated large amounts of starch in the leaves and roots compared with 'M-26', 'Chistock-1' and 'Baleng' rootstocks. Foster *et al.* (2017) reported that the starch concentrations in 'M-9' roots, stems and grafted 'Royal Gala' ('RG') scions were twice as compared to 'RG' homo-grafted trees are in conformity with our study. Dwarfing rootstocks are in a state of sugar depletion and reduced cellular activity despite having large starch reserves. Similarly, in case of carbohydrates, higher accumulations were found in the roots of 'M-9' dwarf rootstock when grafted onto 'Red Fuji'. Previous study has shown that trees grafted onto dwarfing peach rootstocks accumulated higher carbohydrate concentrations compared with trees grafted onto more vigorous rootstocks (Gemma and Iwahori, 1998).

Conclusions

The results of this study demonstrated that the rootstocks alter the dynamics of growth morphology and biochemical characteristics of 'Red Fuji' apple trees. Primary shoot length, number of nodes, internodal length (morphological traits), lower hormonal ratio (IAA+GA_3+ZR)/ABA), lower mineral elements (P, K and Mg) and higher starch contents could be used as main morphological and biochemical indicators for the screening of dwarfing apple rootstocks at early stages of plant growth and development.

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