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Full Length Article

Rootstock Induced Vigour is Associated with Physiological, Biochemical and Molecular Changes in 'Red Fuji' Apple

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Abstract

High-density apple cultivation is favoured by the utilization of dwarfing rootstocks. Evaluation of physiological and biochemical response indicators is essential for early screening of superior dwarfing rootstocks. Here, we investigated the effect of 'M.9', 'M.26', 'Chistock-1'and 'Baleng' rootstocks on growth performance, photosynthesis, biochemical and molecular indices of the 'Red Fuji' scion after 45, 90, and 180 days of grafting (DAG). Apple scion grafted onto 'M.9' rootstock had the lowest plant height and reduced scion diameter and the lowest hydraulic conductance (K_{leaf}), whereas trees grafted onto 'M.26', 'Chistock-1' and 'Baleng' rootstocks were taller and had better scion diameter and hydraulic conductance. In addition, leaf P, K, Mg, Cu, Mn, and Zn concentration was minimum for plants grafted onto 'M.9' rootstock that likely reduced the growth rate. Furthermore, we observed that trees grafted onto 'M.9' rootstock had lower photosynthesis rate (P_n) compared with 'M.26', 'Chistock-1', and 'Baleng' rootstocks. The hormonal and gene expression analysis indicated that there were lower amounts of indole-3-acetic acid (IAA) and zeatin riboside (ZR) and expressions of *YUCCA10a* and *IPT3A* in the leaf and roots of 'M.9' rootstock compared with 'M.26', 'Chistock-1', and 'Baleng' rootstocks. The results of this study can be useful in apple breeding programs to forecast the dwarfing ability at an early stage of growth and development of grafted plants. © 2020 Friends Science Publishers

Keywords: Apple rootstock; Biochemical indicators; Dwarfing; Gene expression; Root morphology; Water status

Introduction

Apple (*Malus* × *domestica* Borkh) is one of the most extensively cultivated fruit trees worldwide (Reig *et al*. 2018). China has taken a prominent position in the apple industry of the world with an average production of 39.70 million tons, and the cultivated area is 2.41 million ha that represents 49% and 46% of the world's share, respectively (FAO 2016). Commercial fruit plants typically consist of two separate genotypes: aerial part (scion) and the underground portion (rootstock) make them complex objects for studying shoot-root communications (Forcada *et al*. 2014; Nawaz *et al*. 2016; Hayat *et al*. 2019). Dwarfing rootstocks are necessary for early and high-density apple plantations (Tworkoski and Fazio 2015). These rootstocks influence many biochemical and physiological parameters of grafted scions (An *et al*. 2017; Adams *et al*. 2018). The physiological mechanisms of how rootstocks influence scion vigour are complex and are not fully understood.

Physiological and biochemical attributes that help the rootstocks to control plant vigour have generally focused on hormone biosynthesis (Hooijdonk *et al*. 2011; Tworkoski and Fazio 2015), nutrient uptake (Amiri *et al*. 2014; Kviklys *et al*. 2017; Khan *et al*. 2020), carbohydrates (Gemma and Iwahori 1998; Foster *et al*. 2017), hydraulic conductance (Basile *et al*. 2003b; Solari and DeJong 2006), phenolic contents (Yıldırım *et al*. 2016) and alteration in anatomical structures (Saeed *et al*. 2010; Tombesi *et al*. 2011); and even within a single species (*Malus pumila*), evidence exists supporting different mechanisms of scion control (Gregory *et al*. 2013). Size-controlling rootstocks could affect the morphological parameters of grafted plants differentially, which includes tree volume, intermodal length, branch composition, trunk cross-sectional area (TCSA) and fruit yield (Gjamovski and Kiprijanovski 2011; Hooijdonk *et al*. 2011; Karlidag *et al*. 2014).

Hormonal regulations have been suggested as a mechanism by which rootstocks modify scion vigour by altered shoot–root–shoot chemical signaling (Pérez-Alfocea *et al*. 2010; Ghanem *et al*. 2011; Song *et al*. 2016;

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Nawaz *et al*. 2017). Lower concentrations of growth-promoting hormones such as auxin, cytokinin and gibberellin and the higher concentration of growth-inhibiting hormones such as abscisic acid in dwarfing rootstocks have been studied in fruit plants. Hormones are well known for controlling tree size (Gregory *et al*. 2013). The rootstock or interstock induced dwarfing effect is due to the alterations in gene expressions linked with hormonal metabolism and transduction, which in turn may regulate the balance of endogenous hormones in scion (Aloni *et al*. 2010; Tworkoski and Fazio 2016). Auxin synthesis primarily occurs in the leaves and roots. YUCCA monooxygenases catalyze the conversion of indole-3-pyruvate to IAA that has a crucial role in auxin biosynthesis (Zhao *et al*. 2001; Won *et al*. 2011). Isopentenyl transferases are the essential enzymes that play a vital role in cytokinin synthesis. Gene expressions of *IPT* directly regulate endogenous cytokinin concentration (Singh *et al*. 1992; Samuelson and Larsson 1993; Takei *et al*. 2004).

Several reports suggest that rootstocks influenced the scion nutrient accumulation in apple and peach plants and the alterations among scion-rootstock communications were linked with nutrient uptake capacity because of their different route structure (Abrisqueta *et al*. 2011; Kviklys *et al*. 2017). Rootstock-scion interactions induce apparent changes in absorption and transport of mineral nutrients to the scion by the alteration of the root structure. However, rootstock performance was not consistent among sites and varied over time (Al-Hinai and Roper 2004).

Rootstocks that reduce scion growth are influence by root morphology, root spreading features, and their association with scion (Ma *et al*. 2013). Dwarfing rootstocks for fruit plants have been used for a long time; the underlying mechanism of rootstock-induced dwarfing is still unknown (Foster *et al*. 2017). The selection of dwarfing rootstocks and screening for superior rootstocks from apple hybrid seedlings are necessary for the breeding of dwarfing rootstocks. This study consists of the measurement of physiological and biochemical indicators for the different plant parts of young apple trees. This study aimed to obtain suitable indices that could be used to predict the degree of dwarfing at the early stage of apple plant growth. Furthermore, we also studied the hormonal levels and the relative expression of hormone-related genes controlling rootstock induced dwarfism in apple plant.

Materials and Methods

Plant materials and culture conditions

The experiment was performed at the Horticultural Laboratory of China Agricultural University, Beijing, China (Longitude 116°24' E, latitude 39°54' N) during March-September, 2018. In March 2018, 1-year old plants of “Red Fuji” grafted onto four rootstocks *viz.*, Malling 9 (‘M.9’, dwarfing), Malling 26 (‘M.26’, semi-dwarfing), ‘Chistock-1’ (*Malus xiaojinensis*, semi-dwarfing) and ‘Baleng’ (*Malus micromalus*, vigorous) were grown in a 30 cm diameter pots containing a blend of garden soil, nursery substrate and sand (3:2:1 v/v) and grown under greenhouse conditions. The plants were irrigated twice on each day, fertilized (1.75 g of 20 N-8.8P-16.6 K w/w/w per tree) on a monthly base. The experiment was carried out in Randomized Complete Block Design (RCBD) and three biological replicates were made (every replicate containing two plants). A total of 120 plants were used in this study.

Determination of morphological characteristics of grafted plants

From May 2018, observations were taken regarding vegetative growth such as plant height (cm), shoot length (cm), leaf number, node number, and intermodal length at 20 days’ interval for each scion-rootstock combination. A digital caliper was used to measure scion diameter (mm) and converted into TCSA using the given formula: TCSA = $\pi(d/2)^2$ (Rahmati *et al*. 2015). The leaf area (cm^2) was measured by using the LI-3100C Area Meter (Li-Cor, Inc., Lincoln, Nebraska, U.S.A.). The measurement of root morphology traits, healthy plants were carefully uprooted and gently washed with water before the leaves dropped in the autumn of 2018. The data for root morphology parameters was assessed by scanning roots using root scanner, and analysis was done by WinRHIZO software (Regent Instruments Inc., Quebec, Canada). The shoot to root ratio was determined by using the formula root fresh weight/shoot fresh weight.

Photosynthetic measurements and hydraulic conductance

A portable photosynthesis system (LI-6400XT, Licor, Inc., Lincoln, NE, USA) was used for measuring net photosynthesis rate (*Pn*), intercellular CO_2 concentration (*Ci*), stomatal conductance (*gs*) and transpiration (*E*), of the fully expanded leaves from 9:00 to 11:30 a.m. after 45, 90 and 180 days after grafting (DAG). All measurements were performed under the given environmental factors: leaf temperature 25 ± 2°C; relative humidity (RH) 65 ± 5%; photosynthetic photon flux 1200 μmol m^{-2} s^{-1} and external CO_2 concentration 400 μmol mol^{-1}. The leaf water potential (ψ_{leaf}) was estimated on uniform fully expanded mature leaves on the same day as gas exchange measurements by the usage of a pressure chamber (Soil-moisture equipment Corp, Santa Barbara, C.A., U.S.A.). Tensiometers were installed at a deepness of 15cm to measure the matric potential (ψ_{soil}) of the pots, as described previously (Qiu *et al*. 2016). The evaporative flux method was used to estimate the Soil -to-leaf hydraulic conductance ($K_{soil-leaf}$). $K_{soil-leaf}$ = E/ $\psi_{soil} - \psi_{leaf}$.

Phytohormone and carbohydrates determinations

Leaf and root samples for endogenous hormones were collected from different scion-rootstock combinations at 45, 90, and 180 DAG. The concentration of zeatin riboside (ZR), indole-3-acetic acid (IAA), abscisic acid (ABA) and gibberellin (GA_3) were measured using Enzyme-linked immunosorbent assay (ELISA) as mentioned by (Zhao *et al*. 2006). The quantification of hormones was calculated based on a standard curves and expressed as ng g^{-1} fresh weight. Soluble sugars and starch were extracted according to the method defined by (Filip *et al*. 2016). The amount of soluble sugars and starch were reported as mg g^{-1} DW.

Measurement of mineral nutrition

Leaf and root samples were harvested at 45, 90, and 180 DAG. Samples were carefully washed with deionized water and then dried at 65°C for 48 h until gaining constant weight. Dried samples were grounded so they can pass through a 40 mesh screen by using a Cyclotec Sample Mill (Cyclotec 1093, Teactor, Hoganas, Sweden). The powder was digested in a mixture of H_2SO_4–H_2O_2. The nitrogen (N) concentration was estimated according to the Kjeldahl method (Nelson and Sommers 1980). The concentrations of other elements of minerals (Mn, P, Ca, Mg, Fe, K, Cu, and Zn) were measured using ICP-MS as per the methods termed by (Masson *et al*. 2010). The results were expressed on a dry matter basis: g kg^{-1} for macro elements (N, P, K, Ca and Mg) and mg kg^{-1} for microelements (Mn, Cu, and Zn).

Gene expression of *YUCCA10A, IPT3A, GA20OX1, NCED1*

The total RNA of leaf and root samples were extracted and purified at 45, 90, and 180 DAG, using an RNAprep Pure Plant Kit (TIANGEN, Beijing, China). Then the quantity and the purity of the RNA were tested using a NanoDrop Spectrophotometer. The first-strand cDNA was synthesized by using the PrimeScript RT reagent kit (Takara, Japan) as per the manufacturer’s directions. Finally, gene-specific primers for RT-PCR were designed by using the NCBI Primer-BLAST. The primer sequence list is given in Table 1. Quantitative qRT-PCR was performed in a 7500 Real-time PCR system (Applied Biosystems, C.A., U.S.A.) using the SYBR Premix Ex Taq kit (Takara, Japan) as per the manufacturer’s directions. qRT-PCR was done using Quantstdio™ 7 Flex Real-Time PCR System, Life Technologies™, Carlsbad, CA, USA. PCR conditions consist of 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and 60°C for 34 s. The standard comparative method was used to calculate relative gene expression (Livak and Schmittgen 2001).

Leaf anatomical characteristics

Six leaves from each treatment combination were selected for the observations of anatomical traits (1 cm × 0.5 cm) and fixed in a 4% paraformaldehyde/0.1 M phosphate buffer. Leaf anatomical traits were measured as described by O'Brien and McCully (1981) and samples were imaged using a BX51TRF fluorescence microscope (Olympus Optical Co., Ltd., Japan). Image-Pro Plus 6.0 software was used to measure the spongy thickness of the leaf, as well as the xylem vessel density and xylem vessel diameter.

Table 1: Primer sequences for the quantification of transcripts by RT-PCR Primer sequences

Primer	Primer sequence
IPT3A Fwd	5- GTGTTCAATCCTCACCGGCA-3
IPT3A Rev	5- GCCGATCACGGCCTAAAATG-3
GA20OX1 Fwd	5- TCTCCGGTGACAAAGAAGCC-3
GA20OX1 Rev	5- GCTCTCCCCTGCTTTCCTTT-3
NCED1 Fwd	5- TCGGAGGACGACGGTTATATTC-3
NCED1 Rev	5- CCATGAAACCCGTAGGGCAC-3
YUCCA10a Fwd	5- CAAGTATCCGATCATTGAC-3
YUCCA10a Rev	5- CCTCTTATGCTGCCTATT-3
B-actin Fwd	5-TGGTGAGGCTCTATTCCAAC -3
B-actin Rev	5-TGGCATATACTCTGGAGGCT-3

Statistical analysis

Data were subjected to the ANOVA (Analysis of variance) in a complete factorial design with three sampling points and four rootstocks using the statistical package SPSS 19.0 (SPSS Inc., Chicago, USA). Mean comparisons among the treatments were made using the least significant difference (LSD) multiple comparison tests at $P < 0.05$.

Results

Phenotypic changes

Rootstocks affected the growth vigour of grafted apple trees (Table 2 and 3). The shoot length of ‘Red Fuji’ apple trees grown onto ‘M.9’ rootstock was lower compared with ‘M.26’, ‘Chistock-1’, and ‘Baleng’ rootstocks (Fig. 1). The most vigorous rootstocks (‘Chistock-1’ and ‘Baleng’) rootstocks resulted in the higher values (5.45 and 6.19 mm respectively) in terms of trunk diameter of the scion, whereas lowest values (5.22 and 5.35 mm respectively) were recorded in ‘M.9’ and ‘M.26’ rootstocks. Leaf area of trees grown with ‘M.9’ and ‘M.26’ rootstocks was lower (38.31 and 42.31 cm^2, respectively) compared with more vigorous rootstocks. ‘Baleng’ rootstock produced longer internodal lengths (1.94 cm), whereas the ‘M.9’ rootstock resulted in the shortest internodal lengths (1.45 cm).

The biomass of ‘Red Fuji’ trees grafted with ‘Baleng’ and ‘Chistock-1’ was markedly higher than vigour controlling rootstocks. Alterations in root length, surface area, root volume, projected area, and the number of root forks and tips among various rootstocks were also noticeable different (Table 2). The ‘M.9’ plants had shorter and smaller roots, whereas the most extensive root length was obtained for the ‘Baleng’ rootstock. The values of root morphological traits including root surface

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

Rootstock	Plant height (cm)	Trunk diameter of the scion (mm)	Internodal length (cm)	Leaf areas (cm^2)	Weight of above-ground part (g)	Weight of root (g
'M.9'	90.25 d	5.222 b	1.45 b	38.31 b	515.68 c	254.73 d
'M.26;	109.5 c	5.352 b	1.817 a	42.31 b	567.33 b	321.86 c
'Chistock-1'	122 b	5.446 b	1.58 b	47.52 a	597.52 b	395.97 b
'Baleng'	140 a	6.194 a	1.941 a	47.83 a	734.48 a	507.51 a

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

Table 3: Root morphological characteristics of 1- year old Red Fuji scion cultivar grafted onto different rootstocks

Rootstock	Average Diameter (mm)	Root Length (cm)	Projected Area (cm^2)	Surface Area (cm^2)	Root Volume (cm^3)	Number of Tips	Number of Forks
'M-9'	1.58 b	2625.96 c	143.61 d	451.17 d	7.36 c	23354.5 c	17870.5 b
'M-26'	2.73 ab	4333.28 c	252.01 c	791.71 c	12.43 bc	27185.5 c	29423.0 b
'Chistock-1'	2.83 ab	11110.13 b	473.97 b	1489.02 b	16.97 ab	75354.5 b	102308.0 a
'Baleng'	3.86 a	16968.46 a	678.00 a	2130.02 a	22.04 a	154002.5 a	134317.5 a

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

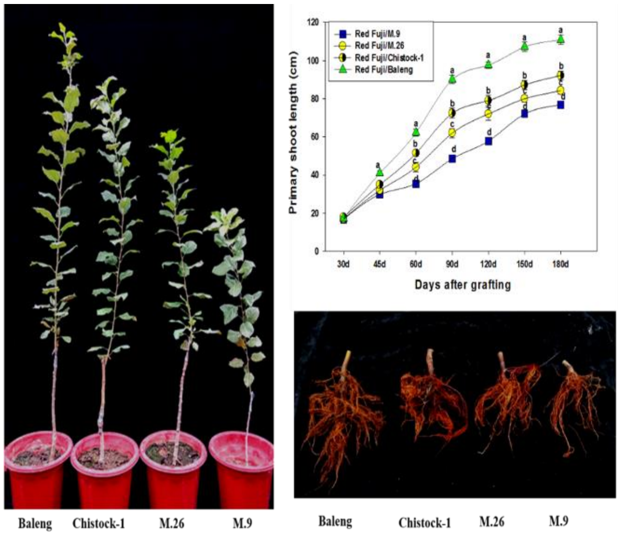

Fig. 1: Phenotypic changes of 'Red Fuji' apple scion grafted onto 'M-9', 'M-26', 'Chistock-1' and 'Baleng' rootstocks. (**A**) Trees grafted on M.9 rootstocks had weakest growth vigour, (**B**) root morphological system, and (**C**) shortest shoot length at different stages of growth and development. Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

area (2130.02 cm^2) projected area (678 cm^2) and the number of forks (134317.5) and tips (154002.5), were also highest in 'Baleng' rootstock compared with dwarfing rootstock ('M.9'). The root-shoot ratios of 'Red Fuji' apple trees grown onto 'Chistock-1' and 'Baleng' (0.662 and 0.690 respectively) rootstocks were also higher than 'M.26' and 'M.9' rootstocks (0.567 and 0.494 respectively).

Hormonal changes

'Red Fuji' apple trees grafted with vigour controlling rootstocks varied in leaf and root IAA contents (Fig. 2). 'Baleng' rootstock enhanced leaf IAA content (88.72 ng g^{-1}) compared with 'M.9' (73.72 ng g^{-1}) and 'M.26' (79.50 ng g^{-1}) rootstocks (Fig. 2A). Root IAA levels (44.21 ng g^{-1}) were lowest for 'M.9' rootstock compared with the 'M.26' (48.68 ng g^{-1}), 'Chistock-1' (60.45 ng g^{-1}) and 'Baleng' (69.65 ng g^{-1}), respectively (Fig. 2B). Leaf ZR levels (9.31 ng g^{-1}) were higher in the 'Red Fuji' apple grafted onto 'Baleng' rootstock, whereas lower ZR levels were obtained for 'M.26' (8.21 ng g^{-1}) and 'M.9' (6.82 ng g^{-1}) rootstocks respectively (Fig. 2C). Root ZR levels (8.61 ng g^{-1}) were also found noticeably higher in 'Baleng' rootstocks compared with 'M.9', 'M.26', and 'Chistock-1' (by 5.81, 7.14 and 7.76 ng g^{-1}, respectively) (Fig. 2D).

Leaf GA_3 levels (10.15 ng g^{-1}) were markedly higher in 'Baleng' rootstocks compared with other size controlling rootstocks utilized in this study (Fig. 2E). In the root, GA_3 levels (7.42 ng g^{-1}) were highest for the 'Baleng' rootstock and lowest for 'M.9', 'M.26', and 'Chistock-1' rootstocks (by 5.78, 5.93 and 6.13 ng g^{-1}), respectively (Fig. 2F). The leaf ABA levels (89.82 ng g^{-1}) were relatively high for 'Red Fuji' grafted onto 'M.26' rootstock followed by 'M.9' rootstock (87.31 ng g^{-1}), whereas 'Baleng' and 'Chistock-1' rootstocks displayed lower levels (by 74.51 and 84.7 ng g^{-1} respectively) (Fig. 2G). The opposite trends were observed for root ABA levels; whereas, the 'M.9' rootstock showed a lower level (44.21 ng g^{-1}) of ABA compared with the three other rootstocks (Fig. 2H).

Gene expression of *YUCCA10a, IPT3A, GA20-ox1, NCED1*

The relative expression of auxin synthesis gene *MdYUCCA10a* was lower in the leaf of 'Red Fuji' apple trees grown onto 'M.9' rootstock compared with trees grown onto 'M.26', 'Red', 'Chistock-1' and 'Baleng' rootstocks at 45, 90, and 180 DAG (Fig. 3A). Similarly, in the roots, the relative expression of the *MdYUCCA10a* gene was reduced in 'M.9' rootstock grafted with 'Red Fuji' compared with other rootstocks (Fig. 3B). The relative expression levels of the *IPT3A* gene were found lower in the roots and leaves of 'M.9' rootstock grafted with 'Red Fuji' apple scion cultivar and higher with 'M.26', 'Chistock-1' and 'Baleng' rootstock (Fig. 3C and 3D). For the *GA20-ox1* gene, 'Red Fuji' apple grafted with 'M.9' and 'M.26'

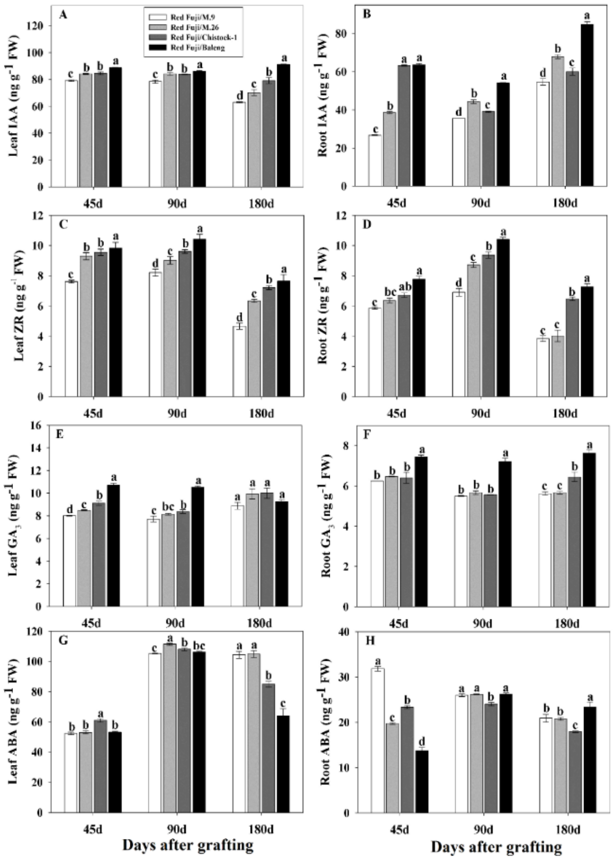

Fig. 2: Endogenous hormone levels in tissue of ‘Red Fuji’ apple grafted onto different rootstocks at different sampling points (x-axis). Endogenous indole-3-acetic acid (IAA) levels of leaf (**A**), and root (**B**); endogenous zeatin riboside (ZR) levels of leaf (**C**), and root (**D**); endogenous gibberellic acid (GA_3) levels of leaf (**E**), and root (**F**); and endogenous abscisic acid (ABA) levels of leaf (**G**) and root (**H**) of ‘Red Fuji’ apple grafted onto different rootstocks. Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

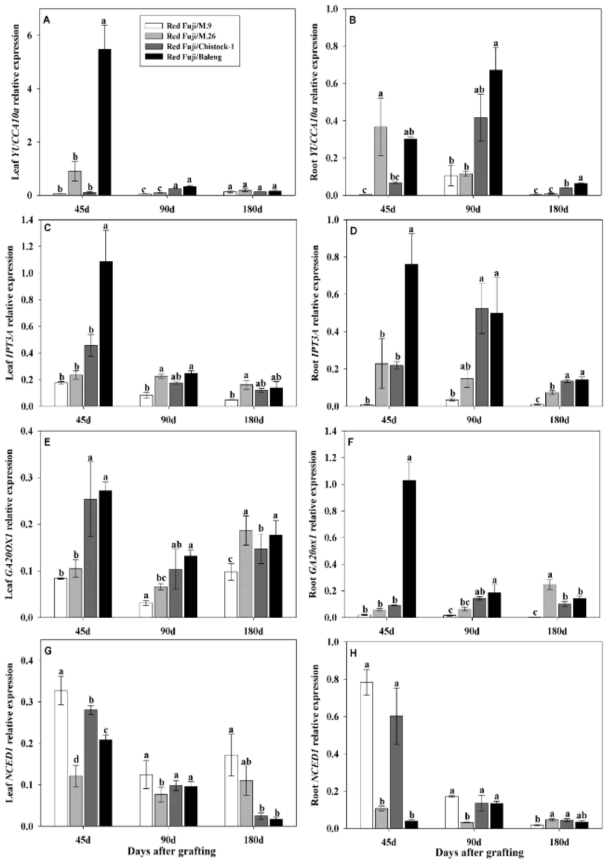

Fig. 3: Relative expression of hormones-related genes in the leaf and roots of ‘Red Fuji’ apple grafted onto different rootstocks at 45, 90 and 180 days after grafting. Different sampling points (x-axis). Relative expression of *MdYUCCA10a* in leaf (**A**), and root (**B**); relative expression of *MdIPT3A* in leaf (**C**), and root (**D**); relative expression of *MdGA20OX1* in leaf (**E**), and root (**F**); and relative expression of *MdNCED1* in leaf (**G**), and root (**H**) of ‘Red Fuji’ apple grafted onto different rootstocks. Error bars represent the standard deviations. Different letters indicate significant differences by LSD ($P \leq 0.05$)

rootstocks had lower relative expressions in their leaves and roots and was comparatively higher with vigorous (‘Baleng’) rootstocks (Fig. 3E and 3F). The expression pattern of *NCED1*gene was higher in the roots and leaves of ‘Red Fuji’/‘M.9’ and decreased with increasing plant size and vigour (Fig. 3G and H).

Mineral nutrient concentrations

‘Red Fuji’ apple cultivar grafted onto different rootstocks behaved differentially regarding nutrient uptake and accumulation in the leaves and roots (Table 5 and 6). ‘Red Fuji’ apple grafted onto ‘M.9’ had lower concentrations for most of the minerals investigated. However, ‘Red Fuji’ grafted onto ‘M.9’ accumulated more N (25.61 g kg^{-1}) in their leaves compared with ‘M.26’ (24.55 g kg^{-1}), ‘Chistock-1’ (24.24 g kg^{-1}) and ‘Baleng’ (25.22 g kg^{-1}) rootstocks, respectively. Trees grafted onto ‘Chistock-1’ rootstock accumulated more P (2.53 g kg^{-1} and 1.938 g kg^{-1}) in the leaves and roots compared with ‘M.9’, ‘M.26’, and ‘Baleng’ rootstocks. ‘Red Fuji’ apple trees grown onto ‘Baleng’ rootstock had higher K concentration (14.95 g kg^{-1}) in the leaves compared with ‘M.9’ (9.78 g kg^{-1}), ‘M.26’ (11.64 g kg^{-1}), and ‘Chistock-1’ (11.73 g kg^{-1}) rootstocks respectively. Additionally, ‘Red Fuji’ apple trees grafted onto ‘Chistock-1’ rootstock had higher Mg concentration (6.03 g kg^{-1}) in their leaves compared with ‘M.9’ (4.52 g kg^{-1}), ‘M.26’ (5.33 g kg^{-1}) and ‘Baleng’ (5.66 g kg^{-1}) rootstocks. Trees were grown onto ‘M.9’ rootstock accumulated less concentration of Cu (5.45 and 5.18 mg kg^{-1}) and Zn (22.39 and 17.52 mg kg^{-1}) in leaves and roots compared with trees grown onto ‘M.26’, ‘Chistock-1’ and ‘Baleng’ rootstocks. ‘Red Fuji’ apple trees grown onto ‘M.9’ rootstock had lower values of leaf Ca concentrations

Table 4: Changes in soluble sugars and starch content in the leaves and roots of ‘Red Fuji’ apple grafted onto different rootstocks measured at 45, 90 and 180 days after grafting (DAG)

Parameters	Rootstocks	Plant tissues	45 DAG	90 DAG	180 DAG	Mean
Starch (mg/g FW)	‘M-9’	Leaf	6.1918 a	5.3927 a	8.3420 a	6.7312 a
		Root	5.6173 a	16.356 a	18.228 a	13.401 a
	‘M-26’	Leaf	5.2677 b	3.4103 b	5.3527 a	4.6769 a
		Root	3.3313 c	6.0290 c	12.954 a	7.4382 b
	‘Chistock-1’	Leaf	4.3130 c	5.1520 a	11.479 a	6.9812 a
		Root	7.8000 c	1.3990 d	6.7803 a	5.3264 b
	‘Baleng’	Leaf	5.1083 b	5.2297 a	4.0835 a	4.8976 a
		Root	8.2217 a	11.242 b	17.963 a	12.476 a
Total soluble sugars (mg/g FW)	‘M-9’	Leaf	39.553 a	32.391 a	28.715 a	33.553 a
		Root	12.528 c	25.702 a	23.717 a	20.649 a
	‘M-26’	Leaf	27.776 b	31.024 ab	27.442 a	28.747 a
		Root	8.0463 d	15.720 b	19.614 a	14.460 a
	‘Chistock-1’	Leaf	25.740 c	29.820 b	28.295 a	27.951 a
		Root	14.288 b	6.3380 c	16.885 a	12.504 a
	‘Baleng’	Leaf	38.088 a	31.755 ab	23.749 a	31.197 a
		Root	17.218 a	15.130 b	21.551 a	14.460 a

Different letters indicate significant differences by LSD ($P \leq 0.05$)

Table 5: Changes in macronutrients content in the leaves and roots of ‘Red Fuji’ apple grafted onto different rootstocks measured at 45, 90 and 180 days after grafting (DAG)

Trait	Rootstock	Plant part	45 DAG	90 DAG	180 DAG	Mean
N (g kg^{-1})	‘M-9’	Leaf	30.36 ab	25.80 a	19.50 c	25.61 a
		Root	18.56 a	10.96 a	12.30 c	14.63 a
	‘M-26’	Leaf	29.30 b	22.66 b	20.76 b	24.55 b
		Root	8.90 c	6.70 b	13.233 b	9.276 c
	‘Chistock-1’	Leaf	31.00 a	21.80 b	20.86 b	24.24 b
		Root	5.233 d	5.50 b	10.60 d	7.111 d
	‘Baleng’	Leaf	31.23 a	23.40 a	22.20 a	25.22 ab
		Root	18.567 a	10.36 a	14.96 a	11.91 b
P (g kg^{-1})	‘M-9’	Leaf	2.715 c	1.703 b	1.901 b	2.106 c
		Root	1.150 c	1.363 b	1.430 d	1.313 d
	‘M-26’	Leaf	2.420 d	2.303 b	2.128 a	2.283 bc
		Root	1.765 b	1.678 a	1.960 b	1.801 b
	‘Chistock-1’	Leaf	2.960 b	2.929 a	1.701 d	2.530 a
		Root	2.033 a	1.296 b	2.486 a	1.938 a
	‘Baleng’	Leaf	3.655 a	1.749 b	1.816 c	2.407 ab
		Root	1.860 a	1.244 b	1.735 c	1.613 c
K (g kg^{-1})	‘M-9’	Leaf	10.06 b	7.233 d	12.33 b	9.778 c
		Root	3.396 b	2.843 b	5.175 b	3.805 c
	‘M-26’	Leaf	8.850 c	12.90 b	13.16 b	11.63 b
		Root	3.593 b	4.570 a	4.905 b	4.356 b
	‘Chistock-1’	Leaf	10.46 b	9.700 c	15.01 a	11.72 b
		Root	2.329 c	3.466 b	5.800 a	3.865 c
	‘Baleng’	Leaf	14.18 a	15.817 a	14.85 a	14.95 a
		Root	4.768 a	4.768 a	4.458 c	4.555 a
Ca (g kg^{-1})	‘M-9’	Leaf	9.050 c	11.50 b	11.96 c	10.89 b
		Root	10.00 ab	7.016 b	8.800 b	8.608 c
	‘M-26’	Leaf	10.20 b	14.58 a	17.98 a	14.25 a
		Root	9.216 b	9.466 a	11.83 a	10.17 a
	‘Chistock-1’	Leaf	10.82 ab	14.76 a	17.83 a	14.47 a
		Root	7.012 c	9.375 a	11.73 a	9.374 b
	‘Baleng’	Leaf	11.90 a	13.23 ab	16.23 b	13.78 a
		Root	10.70 a	8.80 a	8.783 b	9.428 b
Mg (g kg^{-1})	‘M-9’	Leaf	4.313 b	5.216 b	4.051 c	4.527 c
		Root	1.364 b	1.835 b	1.592 d	1.597 c
	‘M-26’	Leaf	2.692 c	5.866 b	6.433 a	5.330 b
		Root	1.465 b	1.686 b	2.136 b	1.7628 b
	‘Chistock-1’	Leaf	5.450 a	7.066 a	5.566 b	6.027 a
		Root	0.986 c	1.895 b	2.281 a	1.721 bc
	‘Baleng’	Leaf	5.475 a	5.900 b	5.616 b	5.663 ab
		Root	2.250 a	2.768 a	1.888 d	2.302 a

Different letters indicate significant differences by LSD ($P \leq 0.05$)

(10.89 mg kg^{-1}) compared with ‘M.26’ (14.25 mg kg^{-1}), ‘Chistock-1’ (14.47 mg kg^{-1}) and ‘Baleng’ (13.78 mg kg^{-1}) rootstocks.

Table 6: Changes in micronutrients content in the leaves and roots of ‘Red Fuji’ apple grafted onto different rootstocks measured at 45, 90 and 180 days after grafting (DAG)

Nutrient	Rootstock	Plant part	45 DAG	90 DAG	180 DAG	Mean
Cu (mg kg^{-1})	‘M-9’	Leaf	3.480 c	6.166 b	6.70 a	5.4489 c
		Root	3.350 d	6.100 b	6.083 b	5.177 c
	‘M-26’	Leaf	7.013 c	7.866 a	5.333 b	6.737 b
		Root	7.413 b	9.383 a	7.316 a	8.037 a
	‘Chistock-1’	Leaf	9.655 a	7.716 a	4.455 c	7.275 a
		Root	5.450 c	11.21 a	6.416 b	7.699 a
	‘Baleng’	Leaf	8.430 b	4.297 c	3.765 d	5.497 c
		Root	9.605 a	6.500 b	4.095 c	6.733 c
Mn (mg kg^{-1})	‘M-9’	Leaf	110.52 a	64.33 a	39.12 b	71.32 a
		Root	22.49 d	43.30 b	34.40 c	33.39 a
	‘M-26’	Leaf	46.77 c	39.40 b	31.66 d	39.29 c
		Root	31.44 a	28.96 bc	14.80 d	25.07 b
	‘Chistock-1’	Leaf	71.27 b	55.83 a	51.66 a	59.59 b
		Root	10.72 d	33.00 ab	38.71 a	27.47 b
	‘Baleng’	Leaf	22.49 d	43.30 b	34.40 c	33.39 d
		Root	28.72 b	25.81 c	16.72 c	23.75 b
Zn (mg kg^{-1})	‘M-9’	Leaf	17.21 d	30.48 b	25.71 b	22.39 c
		Root	12.46 c	14.40 a	19.50 c	17.52 c
	‘M-26’	Leaf	20.91 c	71.50 a	31.80 a	46.03 a
		Root	19.15 b	23.36 a	45.68 a	24.77 a
	‘Chistock-1’	Leaf	25.42 b	26.15 bc	28.58 b	27.50 b
		Root	19.07 b	27.85 a	30.93 b	25.16 a
	‘Baleng’	Leaf	27.80 a	21.62 c	17.06 c	22.95 c
		Root	31.80 a	13.68 b	19.42 c	20.85 b

Different letters indicate significant differences by LSD ($P \leq 0.05$).

Photosynthesis and gas exchange measurements

The data related to gas exchange parameters and hydraulic conductance of ‘Red Fuji’ apple plants grafted onto different rootstocks was affected by the rootstock (Fig. 4). The lowest rate (13.72 mol m^{-2} s^{-1}) of photosynthesis (*Pn*) was observed for trees growing on ‘M.9’ rootstocks, while the highest rate (by 15.29, 16.48 and 17.26 mol. m^{-2} s^{-1}) of photosynthesis (*Pn*) was observed for ‘M.26’ and ‘Chistock-1’ and ‘Baleng’ rootstocks respectively (Fig. 4A). ‘Red Fuji’ apple scion cultivar grafted with ‘M.9’ rootstocks had lower intercellular CO_2 concentration (225.52 mol mol^{-1}) and stomatal conductance (0.12 mol m^{-2} s^{-1}) values compared with ‘M.26’ and ‘Chistock-1’ and ‘Baleng’ rootstocks (Fig. 4B, 4C, 4D). Compared with the ‘Baleng’, ‘Red Fuji’ apple leaves with ‘M.9’, ‘M.26’ and ‘Chistock-1’ showed significant differences for leaf water potential. Trees grown onto ‘M.9’ rootstock had lower (-1.71 MPa) leaf water potential than trees on vigorous ‘Baleng’ rootstock (-1.28 MPa), whereas trees grafted on ‘Chistock-1’ and ‘M.26’ had intermediate values (by -1.489 and -1.563 MPa) respectively (Fig. 4E). Leaf hydraulic conductance (K_{leaf}) showed substantially lower values (1.45 mmol m^{-2} s^{-1} MPa^{-1}) for ‘M.9’ rootstock compared with ‘M.26’ and ‘Chistock-1’ and ‘Baleng’ rootstocks (by 1.99, 2.5641 and 3.101 mmol m^{-2} s^{-1} MPa^{-1}), respectively (Fig. 4F).

Starch and soluble sugars

The starch content of ‘Red Fuji’ apple trees was affected by the use of the different rootstocks (Table 4). In terms of the

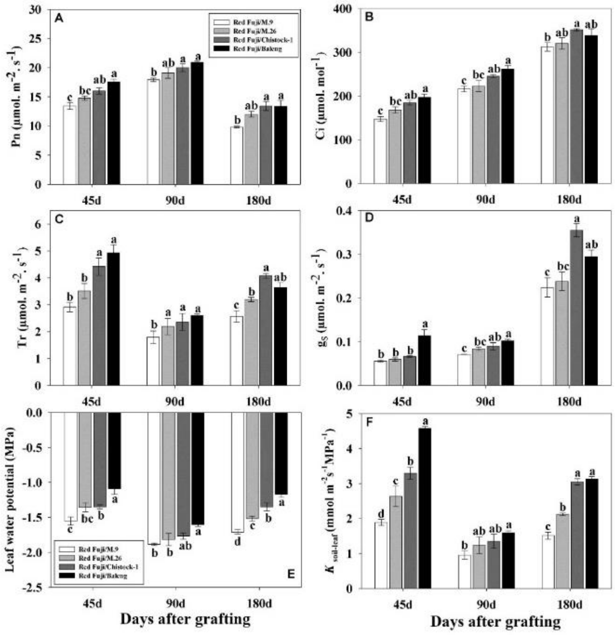

Fig. 4: The Influence of rootstocks on the net photosynthetic rate (**A**), intercellular CO_2 concentration (**B**), transpiration rate (**C**), stomatal conductance (**D**), leaf water potential (**E**) and hydraulic conductance (**F**) of 'Red Fuji' apple leaves at 45, 90 and 180 days after grafting. Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD ($P \leq 0.05$)

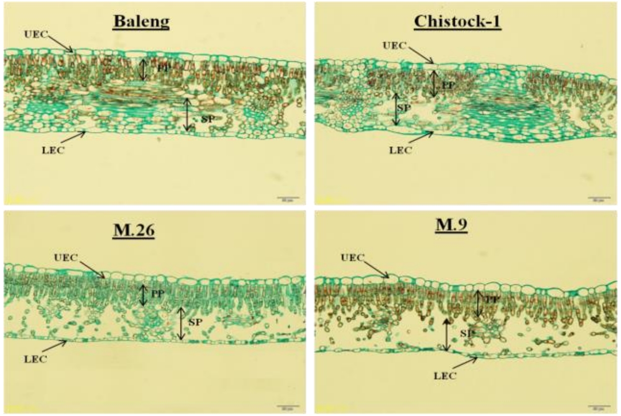

Fig. 5: Leaf anatomical characteristics of 1-year-old Red Fuji scion cultivar grafted onto 'Baleng', 'Chistock-1', 'M-26' and 'M-9' rootstocks

leaf, 'Red Fuji' apple trees grafted with 'M.9' rootstock had higher starch contents but not statistically different from other treatments, whereas trees grafted with 'M.26', 'Chistock-1' and 'Baleng' rootstocks were relatively low starch contents. Root starch contents were also highest (13.4 mg kg^{-1} FW) for 'M.9', followed by 'Baleng' (12.48 mg kg^{-1} FW) rootstock compared with the 'Chistock-1' (5.33 mg kg^{-1} FW) and 'M.26' (7.43 mg kg^{-1} FW) rootstocks. Leaf and root soluble sugars were relatively higher in the 'M.9' rootstock compared with other rootstocks, whereas 'M.26', 'Chistock-1', and 'Baleng' rootstocks displayed lower soluble sugar content.

Leaf anatomical characteristic

The leaf of 'Red Fuji'/ 'M.9' had the lowest average vessel density and average vessel diameter compared with 'M.26', 'Chistock-1', and 'Baleng' rootstocks. Moreover, xylem area, phloem area, and xylem/phloem ratio were highest for 'Baleng' rootstocks, while the other three rootstocks recorded the lowest one (Fig. 5). 'Baleng' rootstock exhibited significantly higher leaf area (47.83 cm^2) and palisade thickness (81.35 um) compared to 'Chistock-1', 'M.26', and 'M.9' rootstocks. Furthermore, palisade/spongy thickness ratio (P/S) result relative to 'M-9' was relatively high (1. 016), followed by 'Chistock-1 (0.913) and 'M-26' (0.996), rootstocks, whereas low values were obtained with 'Baleng' (0.89) rootstocks. Anatomical characteristics revealed the differences in xylem vessel features in the leaf of 'Red Fuji' grafted onto different rootstocks. The number of cortical thickness was also lowest (335.93 um) with 'M.9' rootstock and highest with 'M.26' (343.13 um), 'Chistock-1' (408.69 um) and 'Baleng' (448.91 um) rootstocks, respectively.

Correlation analysis for the rootstock induced morphological and biochemical changes

The correlation among morphological, physiological, and biochemical traits of 'Red Fuji' apple trees grown onto different rootstocks was shown in (Fig. 6). Plant height, shoot length, number of nodes, internodal length, number of root tips, number of root forks, and total root length were positively correlated with leaf water potential, photosynthesis rate, stomatal conductance, and transpiration rate. In comparison, these parameters were negatively correlated with leaf N, Fe and Cu concentrations. Shoot length was negatively correlated with starch contents. Internodal length, trunk diameter of the scion, number of tips, and total root length were also negatively correlated with leaf and root starch contents. Plant height, shoot length, trunk diameter of scion, and intermodal length was positively correlated with leaf P, K, Mg, Mn, IAA and ZR concentrations.

Principal component analysis (PCA) for morphological parameters and biochemical traits

Factor analysis showed that the eigenvalues *(Ei)* of the first three components were all greater than one and represented the main factors (Table 7). Eigenvalues are the variances of the principal components. Because we conducted our principal component analysis on 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

Table 7: Factor analysis of the morphological and biochemical indices of 'Red Fuji' apple grafted onto different rootstocks

Parameters	Component 1	Component 2	Component 3
Plant height	0.936	0.347	0.061
Shoot length	0.932	0.363	0.002
Number of nodes	0.932	0.348	-0.101
Number of leaves	0.73	0.681	-0.058
Internodal length	0.887	0.274	0.372
Trunk diameter of scion	0.751	0.642	0.155
Number of root tips	0.934	0.315	0.168
Number of root forks	0.901	0.342	-0.268
Total root length	0.909	0.415	-0.048
Root surface area	0.925	0.373	-0.064
Root volume	0.956	0.291	-0.036
Leaf area	0.992	0.062	-0.109
Scion fresh weight	0.868	0.487	0.099
Root fresh weight	0.93	0.364	-0.055
Nitrogen (leaf)	-0.435	0.88	-0.192
Nitrogen (root)	-0.608	0.736	0.298
Phosphorus (leaf)	0.871	-0.226	-0.437
Phosphorus (root)	0.588	-0.772	-0.244
Potash (leaf)	0.884	0.416	0.211
Potash (root	0.674	0.303	0.674
Calcium (leaf	0.859	-0.51	0.053
Calcium (root)	0.567	-0.601	0.564
Magnesium (leaf)	0.902	-0.293	-0.316
Magnesium (root)	0.766	0.578	0.28
Iron (leaf)	-0.972	0.227	-0.06
Iron (root)	-0.007	-0.791	0.612
Copper (leaf)	0.307	-0.903	-0.301
Copper (root)	0.66	-0.735	0.154
Manganese (leaf)	0.901	0.349	-0.259
Manganese (root)	0.402	-0.887	0.228
Zinc (leaf)	0.067	-0.787	0.614
Zinc (root)	0.594	-0.804	-0.029
Indole-3-acetic acid (leaf)	0.858	0.49	-0.153
Zeatin riboside (leaf)	1	0.011	-0.026
Gibberellic acid (leaf	-0.273	0.926	0.259
Abscisic acid (leaf)	0.07	0.866	-0.495
Indole-3-acetic acid (root)	0.907	0.369	-0.2
Zeatin riboside (root)	0.992	0.13	-0.009
Gibberellic acid (root)	-0.16	-0.524	0.837
Abscisic acid (root)	-0.598	0.72	0.352
Leaf water potential	0.91	0.316	0.27
Hydraulic conductance	0.985	-0.12	0.125
Photosynthesis rate (P_n)	0.925	-0.326	0.194
Stomatal conductance (G_s)	0.996	-0.089	0.028
Intercellular CO_2 concentration (C_i)	0.954	0.207	0.218
Transpiration rate (E)	0.843	0.532	0.078
Starch (leaf)	-0.452	-0.078	-0.889
Total measured carbohydrates (leaf)	-0.61	0.785	0.109
Starch (root)	-0.374	0.889	0.265
Total measured carbohydrates (root)	-0.558	0.801	0.216
Eigen value	31.663	19.825	5.512
Percent of variance (%)	55.594	34.781	9.670

percent of variance (POV) was 100%, with the amount of information contained in these three factors representing 100% of the total information. Representative indices (with higher weight) of component 1 included 27 evaluation indices, with POVs of 55.6%. Representative indices of component 2 were leaf (GA_3, ABA and Cu) and root (Mn, Zn, and starch) with POVs of 34.81%. Representative indices for component 3 were leaf (starch) and root (GA_3), with POVs of 9.64%. The selection was performed based on the weights of the evaluation indices. Plant height, shoot length and number of nodes (morphological indicator), the number of root tips and root volume (root system morphology index), K_{leaf} (water status), *Pn*, *Gs*, *Ci* (photosynthesis indices), leaf K, Mg, Fe and Mn (minerals), Leaf IAA and GA_3 and root IAA, ZR (hormones) were selected suitable indices for evaluating the dwarfing potential of different apple rootstocks.

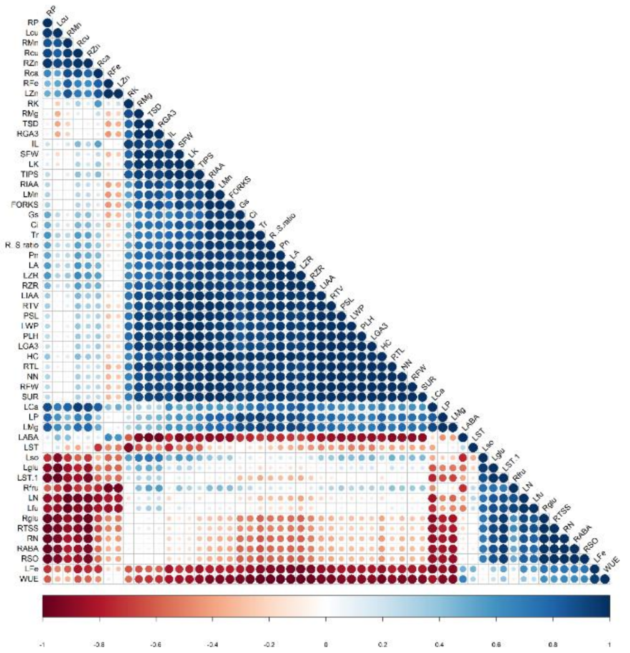

Fig. 6: Correlations of the morphological and biochemical indices of 'Red Fuji' apple grafted onto different rootstocks.
Abbreviations
PLH= plant height, IL= internodal length, NN= number of nodes, TSD= trunk scion diameter, SFW= scion fresh weight, RW= root weight, R:S ratio = root: shoot ratio, TSS= Total soluble sugars, TCL= total carbohydrate in leaf, TCR= total carbohydrate in root, IAA = indole-3-acetic acid, ZR = zeatin riboside, GA_3 = gibberellic acid, ABA = abscisic acid, P = phosphorus, N = nitrogen, K = potassium, Ca = calcium, Mg = magnesium, Fe = iron, Mn = manganese, Zn = Zinc, WUE= water use efficiency, Pn= net photosynthesis rate, E= transpiration rate, Ci= inter cellular CO_2 concentration, Gs = stomatal conductance, LWP= leaf water potential and HC = hydraulic conductance

Discussion

Vigour controlling rootstocks are frequently used in commercial apple culture cause a decrease in tree crown dimension (Foster *et al*. 2017). Various studies have suggested that plants grafted onto vigorous rootstocks have longer shoot lengths, greater trunk diameter of the scion, and vigorous growth (Gjamovski and Kiprijanovski 2011). However, dwarfing rootstocks alter shoot morphology by the decrease in shoot length, node number, and length of sylleptic shoots (Costes *et al*. 2001; Hooijdonk *et al*. 2010). In this study, we observed that the 'Red Fuji' apple grafted onto 'M.9' rootstock induced dwarf size trees, however taller/vigorous rootstock such as 'Chistock-1' and 'Baleng' rootstocks extended the primary shoot length and whole tree architecture (Fig. 1 and Table 2).

Root morphology and architecture of dwarfing rootstocks differ from more vigorous rootstocks (Kumar *et al*. 2018). Root morphological characters can be used for selecting dwarf rootstocks (Luo *et al.* 2014). In this study, we found that the root system of ‘Baleng’ rootstock was stronger with greater root surface area, root volume, projected area, root length, number of tips, and forks of ‘Red Fuji’ apple trees (Table 3). The difference in root morphological traits of rootstocks may be attributed to the genetic alterations among rootstocks (Eissenstat 1991).

A differential ability to synthesize hormones has been associated with rootstock-induced dwarfing mechanism (Hooijdonk *et al.* 2011). Auxin and cytokinin encourage sprouting of axillary buds and tree vigour. Gibberellin favours internodal enlargement, whereas ABA stimulates tree ageing (Yu *et al.* 2012). In the present study, the leaf IAA levels were positively correlated with tree size, being lowest with ‘M.9’, ‘M.26’, and ‘Chistock-1’ rootstocks; however, the highest was recorded in ‘Baleng’ rootstock (Fig. 2A). Hayat *et al.* (2019) reported that ‘Red Fuji’ apple trees grafted onto ‘Baleng’ rootstock were highest in IAA levels. However, trees grafted onto dwarfing rootstocks presented lower values of IAA. Furthermore, ‘Red Fuji’ apple trees grafted with ‘M.9’ rootstock showed lower root IAA levels (Fig. 2B), probably because of lower basipetal IAA transport than vigorous rootstock (Soumelidou *et al.* 1994). Cytokinin is produced in the root portion and transported upwards through the xylem stream (Aloni *et al.* 2005). ZR levels were positively correlated with growth parameters. Trees grafted onto ‘M.9’ rootstock were lowest in leaf and root ZR levels, whereas the highest was found onto ‘M.26’, ‘Chistock-1’, and ‘Baleng’ rootstocks (Fig. 2C and 2D). The reduction of scion growth in ‘Red Fuji’ apple grafted onto ‘M.9’ rootstock is due to limited IAA basipetal transport that, in response, decreased root-derived cytokinin contributed to adequate zeatin shortage in scion potion and consequently suppressed plant growth (Zhang *et al.* 2015). Therefore, a reduced capacity for cytokinin production and movement may be the primary reasons that restrict tree size.

YUCCA monooxygenases catalyzing the conversion of indole-3-pyruvate to IAA has a crucial role in auxin synthesis (Won *et al.* 2011). The expression levels of the *YUCCA10a* gene in leaf and roots were positively correlated with morphological traits and IAA levels of apple trees (Fig. 3A and 3B). The low IAA content in dwarfing rootstock (‘Red Fuji’/‘M.9’) may be attributed to the weaker ability of IAA synthesis in the root and limited root development. The previous study found that ‘Fuji’ apple grafted onto ‘M.9’ rootstock had lower expression levels of IAA synthesis gene *MdYUCCA10a*, possibly induced the lower level of auxin (Song *et al.* 2016). According to another report, *IPT3* shows a vital role, such as the regulator for CK synthesis in *Arabidopsis* (Takei *et al.* 2004; Peng *et al.* 2008). In our trial, relative expression of *IPT3A* gene and ZR content in leaves and roots were much lower in dwarfing rootstock (‘Red Fuji’/‘M.9’) compared with more vigorous rootstocks (Fig. 3C and 3D). This suggests that *IPT3A* actively regulates CK synthesis and movements. Reduced expression of *IPT3A* resulted in reduced ZR synthesis in the roots of dwarfing rootstock (‘Red Fuji’/‘M.9), which led to the decrease of the IAA level in the above-ground part (scion) and reduced growth vigour.

Growth vigour is closely linked with water transport ability and leaf water potential of plants (Jones *et al.* 2010). For ‘M.9’ rootstock, which had stronger size-controlling characteristics, the values of ψ_{leaf} and K_{leaf} were lesser compared with ‘M.26’, ‘Chistock-1’, and ‘Baleng’ rootstock that had vigorous/taller characteristics (Fig. 4E and 4F). These findings were consistent with a previous study (Zhao *et al.* 2016). Therefore, stronger dwarfing effects are caused by hydraulic limitations because the lower capacity of water transport reduced photosynthetic activities, which affect biomass production (Atkinson *et al.* 2003; Webster and Wertheim 2003).

The photosynthetic characteristics of grafted plants are positively correlated with growth vigour (Sabajeviene *et al.* 2006; Nawaz *et al.* 2018). In the current research, plants grafted onto ‘M.9’ rootstock presented lower photosynthetic rates compared with ‘M.26’, ‘Chistock-1’, and ‘Baleng’ rootstocks (Fig. 4A). Numerous studies have reported that the photosynthesis values of trees grown onto size-controlling rootstocks were markedly lower than trees grown on vigorous rootstocks (Fallahi *et al.* 2001; Gonçalves *et al.* 2006; Sotiropoulos 2008). It may be elucidated that size-controlling rootstocks decreased the ability of water transport, prompting the degree of stomatal opening and CO_2 assimilation. Subsequently, the growth vigour of plants are weakened, and the efficiency of photosynthesis declines (Brodribb and Feild 2000). Therefore, photosynthesis rates (P_n) may be used as an indicator to access the dwarfing ability of a rootstock.

Several studies suggest that vigour of rootstock has a substantial effect on the uptake of mineral nutrients in apple trees (Fallahi *et al.* 2001; Kucukyumuk and Erdal 2011). In our study, ‘Red Fuji’ apple trees grafted onto ‘M.9’ rootstock was less efficient than ‘M.26’, ‘Chistock-1’, and ‘Baleng’ rootstocks in the absorption of mineral nutrients. Lower mineral uptake capacity was associated with the root system (total root length, number of tips, and forks) that can directly affect nutrient uptake efficiencies.

Rootstocks influence dynamics of nutrient status may be elucidated as alterations in root distributions, and root functions that affect mineral uptake ability and possible variations in stem and root anatomical structures (Zarrouk *et al.* 2005). The capability of the hydraulic status to supply nutrients through roots to the leaf is related to anatomical characteristics (Atkinson *et al.* 1998). The lower hydraulic conductance will also reduce the rate of nutrient uptake and growth vigour, and this has been suggested as a possible mechanism of scion control (Higgs and Jones 1991).

Xylem characteristics, such as the diameter of vessel and density, considerably influence the growth morphology of scion and rootstocks in grafted fruit plants (Tombesi *et al.* 2011; Chen *et al.* 2015). In the current study, leaf anatomical studies showed that 'Red Fuji'/M-9' had significantly lower xylem vessel diameter and xylem vessel density compared with 'M-26', 'Chistock-1' and 'Baleng' rootstocks (Fig. 5). The lower growth of 'M.9' might be linked with anatomical structures. These characteristics play an essential role in hydraulic status (Hajagos and Végvári 2012; Tombesi *et al*. 2011). Apple and peach dwarfing rootstocks had significantly lower hydraulic conductance compared with semi-dwarfing and taller/stronger rootstocks because dwarfing rootstocks have smaller and fewer xylem vessels (Atkinson *et al*. 2003; Basile *et al*. 2003a). Lower hydraulic conductance of dwarfing rootstock decreases the stem water potential, limits stomata opening, and consequently lesser photosynthetic assimilation (Zorić *et al*. 2012).

Beakbane (1956) reported that apple plants grafted onto size-controlling rootstocks have smaller xylem vessels compared with semi-dwarfing and vigorous rootstocks, and that might lead to reduced hydraulic conductance with causing of enhanced water deficits and therefore reduced growth vigour. Besides, Bauerle *et al*. (2011) reported that the xylem vessel diameter of apple trees grafted onto dwarfing B.9 rootstock decreased compared with MM.111 rootstock in response to drought. The higher accumulation of starch in dwarfing rootstock may be explained by the accumulation of non-structural carbohydrates in leaves inhibits photosynthetic activities that affect plant growth (Araya *et al*. 2006). The previous study showed that trees grafted onto dwarfing citrus rootstocks accumulated higher starch concentrations compared with vigorous rootstocks (Mendel and Cohen 1967). We observed a negative correlation among leaf and root starch contents with morphological traits (including shoot length, number of root tips). Therefore, these indices may be used for selecting dwarfing apple rootstocks.

Conclusion

This study demonstrates that morphological, physiological, and biochemical indices of 'Red Fuji' apples were affected by rootstocks. These indicators can be utilized for the new selection of dwarfing apple rootstocks. The dwarfing rootstock 'M-9' seems to induce lower plant height based on lower shoot length and number of nodes (tree morphological index), the number of root tips and root volume (root system morphology index), P_n, G_s, C_i and K_{leaf} (physiological indices), K, Mg, Fe, Mn and starch (nutrients), IAA, GA_3 (hormones). These are suitable indices for evaluating the dwarfing potential of different rootstocks.

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Author Contributions

Faisal Hayat and Chanpeng Qiu conceived and designed the experiments; Faisal Hayat, Zhou Yanmin and Tian Xue performed the experiments; Summera Asghar and Faisal Hayat analyzed the data; Xuefeng Xu, Ting Wu, Xinzhong Zhang and Wang Yi contributed reagents/materials/analysis tools; Faisal Hayat wrote the paper; Muhammad Azher Nawaz and Zhenhai Han revised the article.

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