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Research Paper

Effects of apple (*Malus* × *domestica*) rootstocks on scion performance and hormone concentration

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ABSTRACT

New apple rootstocks that are fire blight resistant are rapidly becoming available in recent years. Rootstock effect on vigor, yield, return bloom, branch angle, bud break, and hormone profile was assessed using 'Honeycrisp' as the scion cultivar. Three Budagovsky (B.9, B.10 and B.7-20-20), five Geneva (G.814, G.214, G.935, G.41 and G.11) and two Malling (M.9T337 and M.26) rootstocks were used for this trial. G.814 and G.214 were high productive rootstocks, more vigorous than M.9T337 that could be interesting for weak growing cultivars such as 'Honeycrisp'. B.9. B.10, G.41 and G.214 performed pretty similar to M.9T337, but they had slightly higher cumulative yields. High return bloom values were observed on G.935, M.9T337, and G.814, whereas B.72020 was the rootstock with the lowest values. In addition, B.72020 was the stock with more upright branches, while B.10 and G.11 had the flattest. On average, a few rootstocks had a low biennial bearing index, which included B.10 and G.814. Indole-3-acetic acid was the most abundant auxin in the xylem, being B.72020 and B.9 the stocks with higher content, while G.935 had the least. Regarding cytokinins (CK), the highest content was observed on G.11, and the lowest on G.814 and G.214. The highest ABA/CK ratio was observed on B.9, whereas B.72020 had the lowest value. On the other hand, B.72020 had the highest AUX/CK ratio value; and G.11 and G.935 the lowest. Fruit size was associated with high ABA/CK values. Trunk cross sectional area, branch angle, gas exchange variables, and biennial bearing were correlated with auxins and AUX/CK concentrations in xylem. Yield efficiency, crop load, leaf temperature, return bloom and bud break were correlated with ABA, CK, and ABA/CK in xylem. Since G.814, G.935 and G.11 had a very uniform bud break, these rootstocks could be suitable to test in areas where irregular bud break might be a problem. The high levels of endogenous ABA observed in Honeycrisp grafted on B.9 and G.11 suggests the possibility of these rootstocks tolerating drought stress by slowing evapotranspiration of the scion.

1. Introduction

Planting a new apple (*Malus* × *domestica* Borkh.) orchard nowadays encompasses a 15–25 year commitment. Cultivar and rootstock selection, and choice of training system are a few among other important key decisions that will affect an orchard's economic and environmental viability. Increasing light interception and improving its distribution through the canopy, planting with narrower spacing, increasing within row density, can boost quality and produce higher orchard yields (Lakso and Robinson, 2014; Palmer, 2011; Robinson, 2010; Tustin and van Hooijdonk, 2014). However, it has been shown that as planting density is increased there is a point where additional economic and productivity benefits decrease with each additional tree (Robinson, 2008; Robinson et al., 2007). The concept of high density orchards relies on high early yields are needed to pay back the initial intensive investment in trees and high density support infrastructure (posts, wire, irrigation, etc.). Tree vigor and its size and shape affect not only yield and precocity, but management costs (Giovannini and Liverani, 2005; Wertheim et al., 2001). Dwarfing apple rootstocks have made possible the transition of entire industries to higher tree density and training systems over the last 50 years. Although the transition has been mostly beneficial, there is a serious threat to high-density apple orchards, since many of them are planted on dwarfing rootstocks as M.9 and M.26 that are highly susceptible to the bacterial disease fire blight (*Erwinia amylovora* Burill), thus, limiting the establishment of new plantings in fire blight prone areas (Norelli et al., 2003; Russo et al., 2007). Climate change exemplified by the increase in average winter temperatures during the last decades, and insufficient chilling units accumulated in warm climates like Brazil, might hinder appropriate bud break on apple trees, compromising their productivity (Flaishman et al., 2010; Oukabli et al., 2003; Petri et al., 2012).

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Changing climate and increased disease pressure underscore an urgent need for new, highly productive apple rootstocks that are resistant biotic and abiotic stresses to fire blight and are adapted to the climatic stresses common in apple production areas of the world. A number of new apple rootstocks that are resistant to fire blight and other biotic stresses are rapidly becoming available. The Cornell University − USDA-ARS apple rootstock breeding program has developed rootstock genotypes which are resistant to fire blight and crown rot (*Phytophthora* spp.) (Cummins and Aldwinckle, 1983; Fazio et al., 2015). Moreover, the dwarfing rootstock B.9, from the Budagovsky breeding program, has recently been reported to have high degree of field resistance to fire blight (Russo et al., 2008a; Russo et al., 2008b). Dwarfing and semi-dwarfing fire blight resistant rootstocks from various breeding programs have been widely tested with 'Gala', 'Fuji', 'Golden Delicious' and 'McIntosh' cultivars through the NC-140 coordinated rootstock testing system (Autio et al., 2013; Autio et al., 2011; Marini et al., 2006a; Marini et al., 2014) resulting in the identification of several rootstocks amenable to widespread cultivation of those varieties. Still, the popularity of several weak-growing cultivars such as 'Honeycrisp' requires a re-evaluation of promising rootstocks when the scion cultivar is not vigorous (Robinson et al., 2011b).

Tree vigor and yield are the main variables assessed when testing rootstocks. However, other variables like branch angle, biennial bearing, and hormone profile may also play a role with the performance of these rootstocks (Fazio and Robinson, 2008; Hollender and Dardick, 2015; Lauri and Lespinasse, 2001; Tworkoski and Miller, 2007; Van Hooijdonk et al., 2011).

Plant hormones are organic substances that have the ability to affect physiological processes at concentrations far below those where either nutrients or vitamins would affect these processes (Davies, 2004). The role of hormones in regulating physiological processes and influence on biennial bearing has been widely described (Davies, 2004; Jackson, 2003; McLaughlin and Greene, 1991b; Ramírez et al., 2004). Bud break and dormancy release has been reported to be regulated by cytokinins (Saure, 1985; Young, 1989), and gibberellins (Lavender et al., 1973). Other hormones like auxins have been associated in controlling the vigor of the trees (Sorce et al., 2007; Sorce et al., 2006). Cytokinins are produced in the root meristems, where they travel all the way up the stem through the xylem (Davies, 2004). Auxins are primarily synthetized in leaf primordia (Davies, 2004), but Ljung et al. (2005) reported roots to contain multiple auxins sources as well. Phenotypic diversity for endogenous hormone production has been demonstrated (Tworkoski and Fazio, 2016; Tworkoski et al., 2016) hence, we may consider that different rootstocks will induce different hormone profiles to the scion, affecting its overall performance.

The collection and analysis of xylem exudates (Bangerth, 2008), has enabled the observation of hormone fluxes (Kamboj and Quinlan, 1997) and profiles on young apple trees grafted on Malling rootstocks (Tworkoski and Fazio, 2016; Tworkoski and Miller, 2007), suggesting that some physiological processes leading to dwarfing and productivity are slow and may require time for clear manifestation.

The aim of this study was to assess the performance of different rootstocks at a mature orchard production stage. Interaction among tree growth variables and how rootstocks may modify the vigor, yield, return bloom, branch angle, bud break, and hormone profile of the weak biennial bearing scion cultivar 'Honeycrisp' was examined.

2. Materials and methods

2.1. Trees and design

A rootstock trial was planted in 2010 at the New York State Agricultural Experiment Station (Geneva, NY, USA), using 'Honeycrisp' as the scion cultivar. Trees were planted in a randomized complete block design, with 4 replications and with each block containing 2–3 trees of each rootstock. Blocking was done by initial tree diameter. Tree spacing was 1.2 m × 3.5 m. Rootstocks included 2 Malling: M.26 EMLA and M.9T337; 3 Budagovsky: B.7-20-20, B.10 and B.9; and 5 Cornell Geneva: G.11, G.41, G.935, G.214, and G.814.

2.2. Growth, yield and performance measurements

Tree survival, trunk circumference (30 cm above the graft union), number of suckers, yield and number of fruits were assessed every year. Trunk-cross-sectional area (TCA) and fruit size were then calculated. In 2016, crotch angle from the trunk (0° = flat, 90° = upright) was measured on 15 branches per tree and two trees per rep. Return bloom was measured in the spring of 2016 by dividing the number of flowering spurs per total number of spurs on 4 branches per tree and two trees per rep. Biennial bearing was calculated as follow: (year 1 yield) − (year 2 yield)/(year 1 yield + year 2 yield), where 0 indicates no alternate bearing and 1 complete alternate bearing. Percentage of bud-break on terminal buds was recorded every two days, with any bud showing 3 mm green tip considered broken (Belding and Young, 1989).

2.3. Physiological measurements

In 2016, carbon assimilation (A, μmol CO2 m^{-2} leaf area s^{-1}), transpiration (E, mmol H2O m^{-2}leaf area s^{-1}), and stomatal conductance (gs, mmol m^{-2} s^{-1}) were measured on two leaves on two trees per rep (16 leaves per treatment) on alternated days during two weeks (CIRAS-1 PP Systems, Haverhill, MA) (Tworkoski and Fazio, 2011). Average gas exchange values were calculated.

2.4. Hormone analysis

In 2016, xylem exudates were extracted at bloom, coinciding with the highest cytokinin concentration in xylem (Belding and Young, 1989), from two one-year-old branches from two trees per rep, using a similar methodology described by Sorce et al. (2007) and a pressure chamber (600 EXP Super Chamber, PMS Instrument Company, Albany, Oregon, USA). Samples from each replicate were pooled to produce an experimental unit and were stored at −80 °C until analyzed. The identification and quantification of the hormones were done by UPLC ESI–MS/MS (Lulsdorf et al., 2013; Zaharia et al., 2005) at the National Research Council Plant Biotechnology Institute (110 Gymnasium Place, Saskatoon, Saskatchewan, S7N0W9, Canada).

2.5. Data analysis

Response variables were modeled using linear mixed effect models. Mixed models including rootstock as fixed factor and block as a random factor were built to separate treatment effects for the TCA, fruit size, yield, yield efficiency, crop load, return bloom, branch angle, biennial bearing index, and hormone content. Yield was included as covariate to adjust fruit size. A mixed model including rootstock, day, and rootstock x day as fixed factors and tree nested to block and block as random factors was built to separate the treatment effects for bud break. For all the models, when the main effect (rootstock) was significant, pairwise comparisons between the rootstocks were made by LS means Student *t*-test. *P* values were corrected using the false discovery rate (FDR) to control for multiple comparisons (Benjamini and Hochberg, 1995). Adjusted *P* values ≤ 0.05 were considered significant. Residual analysis was performed to insure that model assumptions were met.

Multivariate projection methods (Principal Component Analysis, PCA) were applied to simultaneously analyze performance and physiological variables. For this purpose, we used the following variables: Cumulative TCA, yield (2015), fruit number (2015), cumulative yield and fruit number, fruit size (2015), average branch angle and biennial bearing index, return bloom (2016), yield efficiency (2015), crop load (2015), cumulative yield efficiency and crop load, carbon assimilation, transpiration and stomatal conductance. All the variables were analyzed in the same matrix.

Table 1

Trunk cross sectional area (TCA), adjusted fruit size, cumulative yield, cumulative yield efficiency, cumulative crop load, yield, return bloom, branch angle, and biennial bearing indexes for each different rootstock. Cumulated values are from 2010 to 2015. Within each response variable mean values followed by the same letter do not differ at $P \le 0.05$. Rootstocks are ranked by TCA.

Rootstock	TCA (cm^2)	Adj. fruit size (g)	Cum. yield (kg/tree)	Cum. yield eff. (kg/cm^2 TCA)	Cum. crop load (#/cm^2 TCA)	2015 Yield (kg/tree)	2016 Return bloom (%)	Branch angle (Degrees)	Biennial bearing index (2012–2013)	Biennial bearing index (2013–2014)	Biennial bearing index (2014–2015)	Mean biennial bearing index
B.9	7 g	217 d	41 e	5.7 a	26.2 a	17 e	38 abc	15 b	0.27 d	0.64	0.69 abc	0.53 ab
G.11	12 f	281 abc	63 cd	5.3 ab	20.6 bc	32 bcd	48 ab	10 b	0.41 abcd	0.60	0.61 abc	0.54 ab
B.10	14 ef	264 bc	72 abc	5.2 abc	21.3 b	29 bcd	40 abc	10 b	0.16 d	0.36	0.45 bc	0.32 b
M.26	14 def	256 bc	52 de	3.7 e	15.3 d	24 de	33 bc	14 b	0.63 ab	0.68	0.55 abc	0.61 ab
G.41	15 de	288 ab	67 bc	4.7 abcd	17.9 bcd	34 bcd	34 bc	13 b	0.29 cd	0.63	0.76 ab	0.56 ab
M.9T337	15 de	280 abc	63 cd	4.6 bcde	17.9 bcd	27 cd	55 ab	11 b	0.33 bcd	0.57	0.46 bc	0.45 ab
G.214	17 bcde	256 c	76 abc	4.6 bcde	18.3 bcd	37 abc	27 bc	12 b	0.42 abcd	0.50	0.66 abc	0.53 ab
G.935	18 bc	257 bc	66 bcd	3.8 de	16.2 cd	29 bcd	59 ab	12 b	0.57 abc	0.70	0.58 abc	0.61 ab
G.814	19 b	271 abc	83 a	4.4 bcde	17.6 bcd	34 bcd	51 ab	14 b	0.33 bcd	0.44	0.33 c	0.37 ab
B.72020	47 a	293 ab	66 bcd	1.4 f	4.9 e	45 a	13 c	24 a	0.66 a	0.59	0.84 a	0.70 a
P value	< 0.0001	0.0013	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0104	< 0.0001	0.0003	NS	0.0053	0.0395

A two-way hierarchical cluster using the Ward method was built in order to classify the rootstocks based on all the variables analyzed (growth and performance, physiological, and hormone assessments). All the data were standardized before analysis. Data were analyzed using the JMP statistical software package (Version 12; SAS Institute Inc., Cary, North Carolina).

3. Results

B.72020 was significantly more vigorous than all other stocks (Table 1). Then, a lesser vigorous group comprised G.814, G.935 and G.214. A smaller vigorous group included M.9T337, G.41, M.26 and B.10. A slightly smaller stock was G.11, followed by B.9, which was the most dwarfing stock of the trial. No significant differences were observed regarding tree survival (data not shown).

Fruit size was excellent on most of the rootstocks, with an average weight of 270 g (Table 1). Larger fruits were observed on B.72020, G.41, G.11 and M.9T337, whereas the smallest fruits were observed on B.9.

Over the last 6 years, G.814 with 83 kg tree^{-1} had the highest cumulative yield, followed by G.214, and B.10 (Table 1). Lowest yields were observed on B.9 and M.26. On the other hand, the most yield efficient stock of the trial was B.9, followed by G.11, B.10 and G.41. Lowest yield efficiency and crop load rates were observed on B.72020, far below than the rest of the stocks, and then M.26 and G.935.

High return bloom values were observed on G.935, M.9T337, and G.814; followed by G.11, B.10, and B.9 (Table 1). B.72020 was the rootstock with least return bloom (13%), but the one, together with G.214, G.814, G.41 and G.11 with highest yields in the previous year. In addition, B.72020 was the stock with more upright branches, whereas B.10 and G.11 had the flattest (Table 1).

The biennial bearing index was calculated for each stock for 2012–2013, 2013–2014, and 2014–2015 (Table 1). Some rootstocks like B.9, G.11, and G.41 had a low biennial bearing index in some years, and a higher index in other years. On average, a few rootstocks had a low biennial bearing index, which included B.10 and G.814.

A PCA analysis was built to study the relation among the different variables that are usually assessed when testing rootstocks (Fig. 1). The first two principal components (PCs) were able to explain 57% (38% PC1 and 19% PC2) of the overall variance. Yield, fruit number and cumulated of both were highly correlated among them, and highly negatively correlated with return bloom. On the other hand, yield efficiency, crop load, and their cumulated values were highly negatively correlated with the branch angle, which was very positively correlated with carbon assimilation. The most important variable for the definition of the first PC was the TCA, which was also positively correlated with stomatal conductance, carbon assimilation and branch angle. Fruit size and transpiration were extremely correlated.

Higher percentage ($> 55\%$) of buds broke at the same time on March 30th on G.935, G.814 and G.11 rootstocks (Fig. 2). Statistical differences were observed for G.935 compared to G.11, G.214, G.41, M.26, M.9T337, B.10, B.9 and B.72020 (FDR Adjusted P value ≤ 0.05). Statistical differences were also observed among G.814 versus B.72020, M.9T337 and B.9; and between G.11 and B.72020 and B.9 (FDR Adjusted P value ≤ 0.05). B.72020 was the stock on which less buds broke at the same time (FDR Adjusted P value ≤ 0.05). Bud break on most of the rootstocks tended to equalize as the season progressed, with the exception of B.72020.

Indole-3-acetic acid (IAA) was the most abundant auxin in the xylem extracts, being B.72020 and B.9 the stocks with higher content, while G.935 had the least (Table 2). Other IAA conjugates such as *N*-(Indole-3-yl-acetyl)-alanine (IAA-Ala), *N*-(Indole-3-yl-acetyl)-aspartic acid (IAA-Asp), *N*-(Indole-3-yl-acetyl)-glutamic acid (IAA-Glu), and *N*-(Indole-3-yl-acetyl)-leucine (IAA-Leu) were also present in much lower concentration, but no significant differences were observed among rootstocks.

Regarding gibberellins (GA), results indicate that GAs on the 13-hydroxylation pathway (GA53 → GA44 → GA19 → GA20(→GA29) → GA1 → GA8) were more abundant than those on the non-hydroxylation (GA12 → GA15 → GA24 → GA9(→GA51) → GA4 → GA34) pathway present in those samples. The presence of GA34 and GA8 indicates that biologically active GA4 and GA1 respectively, must have been previously produced (Table 2). Significant differences among stocks were only observed for GA53. B.9 had the highest content, whereas G.41 had in the lowest.

High presence of biologically active abscisic acid (ABA) and most of its catabolites in all rootstocks was observed (Table 2). The main catabolism pathways were through 8′-hydroxylation (resulting in phaseic acid, PA, which is further reduced to dihydrophaseic acid, DPA), followed by conjugation (resulting in abscisic acid glucose ester, ABAGE). Other secondary catabolism pathways were also present, namely 7′- and 9′-hydroxylation, resulting in 7′-Hydroxy-abscisic acid (7′OH-ABA) and neo-phaseic acid (neo-PA), respectively. *Trans*-ABA was formed through the isomerization of natural ABA under UV light exposure. The highest overall ABA content was observed on G.11 and B.9, whereas B.72020 and G.814 were the least abundant in ABA and ABA catabolites.

Among all the bioactive free base cytokinins − Zeatin (Z), dihydrozeatin (dhZ), Isopentenyladenine (iP) − only Z (*trans*-isomer) and iP were found in most samples (Table 2). However, the biosynthetic precursors Zeatin riboside, ZR (both *cis*- and *trans*-isomers, the latest significantly more abundant), dhZR and Isopentenyladenosine (iPR) were detected as well. Differences among rootstocks were mainly observed

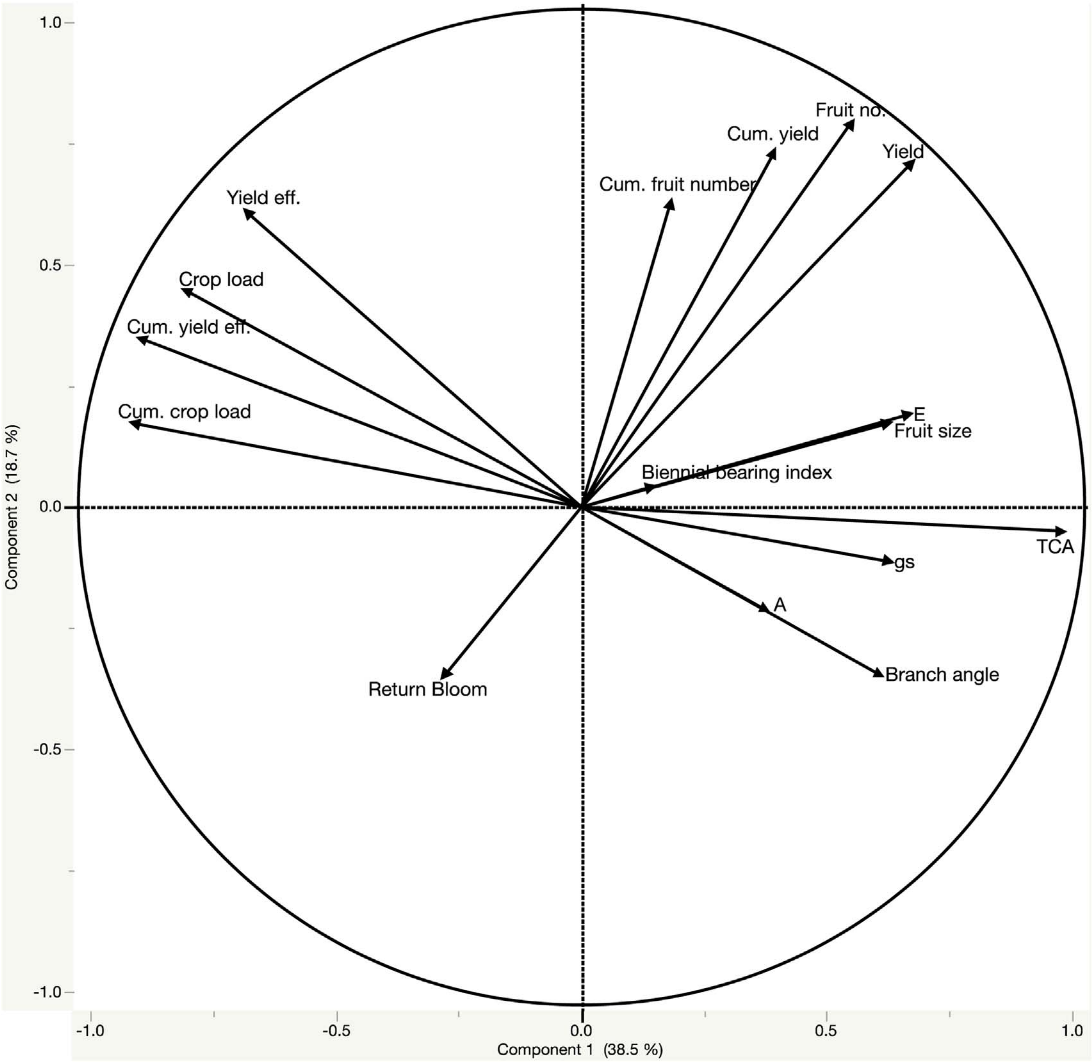
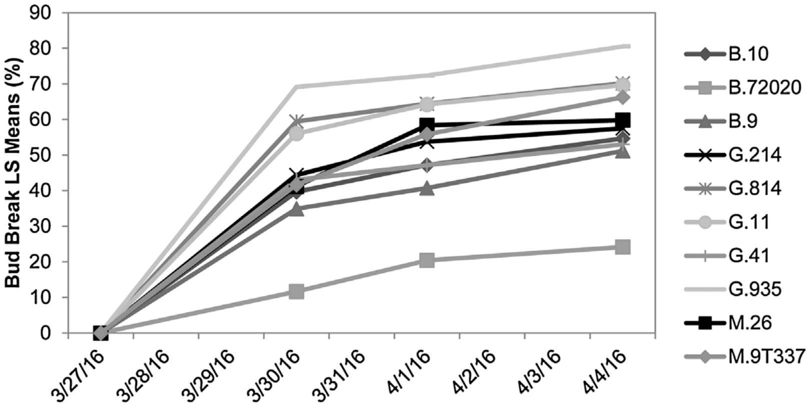


Fig. 1. Variable loadings represented in the plane defined by the first two principal components (PCA analysis). Variables are: Cumulative fruit number, cumulative yield, fruit number, yield, mean biennial bearing index, fruit size, transpiration (E), trunk cross sectional area (TCA), stomatal conductance (gs), carbon assimilation (A), branch angle, return bloom, cumulative crop load, cumulative yield efficiency, crop load and yield efficiency.



Source	Nparm	DF	DFDen	F Ratio	Prob > F
Rootstock	9	9	114.3	6.1766	<.0001
Day	3	3	265.8	354.8273	<.0001
Rootstock*Day	27	27	265.8	3.1584	<.0001

Fig. 2. Bud break (%) for the different rootstocks along the season.

Table 2
Hormone profile in xylem content of the different rootstocks. Analyzed hormones are auxins and associated metabolites indole-3-acetic acid, N-(Indole-3-yl-acetyl)-alanine, N-(Indole-3-yl-acetyl)-aspartic acid, N-(Indole-3-yl-acetyl)-glutamic acid, N-(Indole-3-yl-acetyl)-leucine, Indole-3-butyric acid; gibberellins (GA) 1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 34, 44, 51, and 53; *cis*-abscisic acid and metabolites dihydrophaseic acid, abscisic acid glucose ester, phaseic acid, 7′-Hydroxy-abscisic acid, neo-Phaseic acid, *trans*-Abscisic acid; cytokinins and metabolites (trans) Zeatin-O-glucoside, (cis) Zeatin-O-glucoside, trans Zeatin, (cis) Zeatin, dihydrozeatin, trans Zeatin riboside, (cis) Zeatin riboside, dihydrozeatin riboside, Isopentenyladenine, Isopentenyladenosine. Within each response variable mean values followed by the same letter do not differ at $P \le 0.05$.

Rootstock	Auxins (ng/g FW)							Gibberellins (ng/g FW)														
	IAA	IAA-AIa	IAA-Asp	IAA-Glu	IAA-Leu	IBA	Total Auxins	GA1	GA3	GA4	GA7	GA8	GA9	GA19	GA20	GA24	GA29	GA34	GA44	GA51	GA53	Total Gibberellins
B.10	3.3 b	2.00E-02	0.02	2.00E-02	−6.94E-18	0.0	3.4 b	0.0	2.00E-02	0.08	0.06	0.08	0.0	0.51	0.04	0.17	0.12	0.10	0.12	0.00	0.4 ab	1.70
B.72020	9.1 a	3.47E-18	0.14	2.00E-02	6.00E-02	0.0	9.3 a	0.0	2.00E-02	0.04	0.11	0.09	0.0	0.42	0.04	0.18	0.14	0.15	0.25	0.00	0.5 ab	1.90
B.9	5.3 ab	9.00E-02	0.09	4.50E-02	−6.94E-18	0.0	5.6 ab	0.0	3.12E-17	0.02	0.11	0.17	0.0	0.58	0.00	0.18	0.00	0.15	0.19	0.00	1.2 a	2.55
G.214	3.2 b	−3.47E-18	0.07	4.50E-02	−6.94E-18	0.0	3.3 b	0.0	3.12E-17	0.11	0.07	0.14	0.0	0.52	0.07	0.17	0.00	0.12	0.16	0.00	0.6 ab	1.97
G.814	4.9 b	−3.47E-18	0.00	−6.94E-18	4.50E-02	0.0	5.0 b	0.0	6.50E-02	0.16	0.07	0.09	0.0	0.39	0.00	0.16	0.07	0.18	0.15	0.14	0.5 ab	1.94
G.11	3.9 b	−6.94E-18	0.14	4.50E-02	−6.94E-18	0.0	4.1 b	0.0	4.50E-02	0.11	0.13	0.07	0.0	0.66	0.11	0.27	0.17	0.17	0.21	0.05	0.8 ab	2.79
G.41	3.4 b	−3.47E-18	0.11	4.50E-02	4.50E-02	0.0	3.6 b	0.0	4.50E-02	0.13	0.13	0.14	0.0	0.51	0.09	0.25	0.10	0.17	0.19	0.07	0.3 b	2.11
G.935	3.0 b	4.50E-02	0.09	6.50E-02	4.50E-02	0.0	3.3 b	0.0	6.50E-02	0.09	0.09	0.07	0.0	0.55	0.04	0.22	0.11	0.14	0.29	0.00	0.6 ab	2.29
M.26	3.6 b	2.00E-02	0.17	0.00E+00	−3.47E-18	0.0	3.8 b	0.0	2.00E-02	0.11	0.11	0.11	0.0	0.49	0.07	0.15	0.06	0.15	0.00	0.00	0.7 ab	1.97
M.9T337	4.4 b	4.50E-02	0.05	4.50E-02	4.50E-02	0.0	4.6 b	0.0	3.12E-17	0.14	0.14	0.21	0.0	0.63	0.05	0.21	0.00	0.20	0.22	0.07	0.6 ab	2.43
*P*value	0.0082	NS	NS	NS	NS	NS	0.0077	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.0391	NS

	ABA and ABA metabolites (ng/g FW)								Cytokinins (ng/g FW)													
	ABA	DPA	ABAGE	PA	7′OH-ABA	neo-PA	t-ABA	Total ABA metabolites	t-ZOG	c-ZOG	t-Z	c-Z	dhZ	t-ZR	c-ZR	dhZR	iP	iPR	Total Cytokinins	ABA/CK	AUX/CK	GA/CK
B.10	54 ab	8 bc	1.9	60 ab	0.13 ab	0.21 ab	0.9	125 ab	1.30E-18	9.50E-03	0.044	4.34E-19	−1.13E-17	1.0 ab	0.13	0.07	0.036	0.73	1.98	64 ab	2.3 b	0.9
B.72020	26 b	4 c	1.3	28 b	0.11 b	0.14 b	0.3	60 b	2.17E-18	1.73E-18	0.033	8.67E-19	1.65E-02	0.7 b	0.09	0.11	0.033	0.71	1.69	35 b	5.7 a	1.1
B.9	72 a	24 a	2.1	116 ab	0.24 ab	0.39 a	1.7	217 a	9.50E-03	1.73E-18	0.041	9.50E-03	−1.21E-17	0.8 ab	0.16	0.05	0.040	0.85	1.98	111 a	3.0 ab	1.4
G.214	44 ab	10 bc	1.6	60 ab	0.16 ab	0.26 ab	1.0	117 ab	1.30E-18	1.73E-18	0.042	4.34E-19	7.00E-03	0.5 b	0.09	0.06	0.037	0.98	1.75	79 ab	2.0 b	1.3
G.814	30 b	8 bc	1.7	26 b	0.11 b	0.20 ab	0.4	67 b	4.34E-19	9.50E-03	0.031	4.34E-19	9.50E-03	0.5 b	0.10	0.10	0.041	0.79	1.59	47 b	3.0 ab	1.4
G.11	70 a	15 ab	1.9	129 a	0.29 a	0.41 a	1.6	219 a	9.50E-03	9.50E-03	0.042	4.34E-19	1.20E-02	1.8 a	0.19	0.08	0.041	1.14	3.28	67 ab	1.3 b	0.9
G.41	53 ab	9 bc	1.3	78 ab	0.19 ab	0.29 ab	1.4	142 ab	1.30E-18	1.73E-18	0.053	9.50E-03	7.00E-03	1.2 ab	0.13	0.08	0.033	0.70	2.23	68 ab	2.8 b	1.4
G.935	45 ab	11 bc	1.4	78 ab	0.20 ab	0.25 ab	1.1	137 ab	1.30E-18	9.50E-03	0.044	4.34E-19	7.00E-03	1.3 ab	0.16	0.12	0.036	1.13	2.77	50 b	1.3 b	0.8
M.26	48 ab	12 bc	1.4	67 ab	0.17 ab	0.23 ab	1.0	130 ab	1.73E-18	2.17E-18	0.033	6.51E-19	1.65E-02	0.9 ab	0.14	0.07	0.041	0.79	1.95	66 ab	1.9 b	1.1
M.9T337	43 ab	9 bc	2.3	50 ab	0.18 ab	0.30 ab	0.7	106 ab	1.90E-02	−2.17E-18	0.043	−2.17E-19	−1.04E-17	1.2 ab	0.14	0.09	0.043	1.14	2.63	42 b	2.1 b	1.1
*P*value	0.0254	0.002	NS	0.034	0.0296	0.0293	NS	0.0213	NS	NS	NS	NS	NS	0.0101	0.0431	0.0327	NS	NS	0.0386	0.0287	0.0074	NS

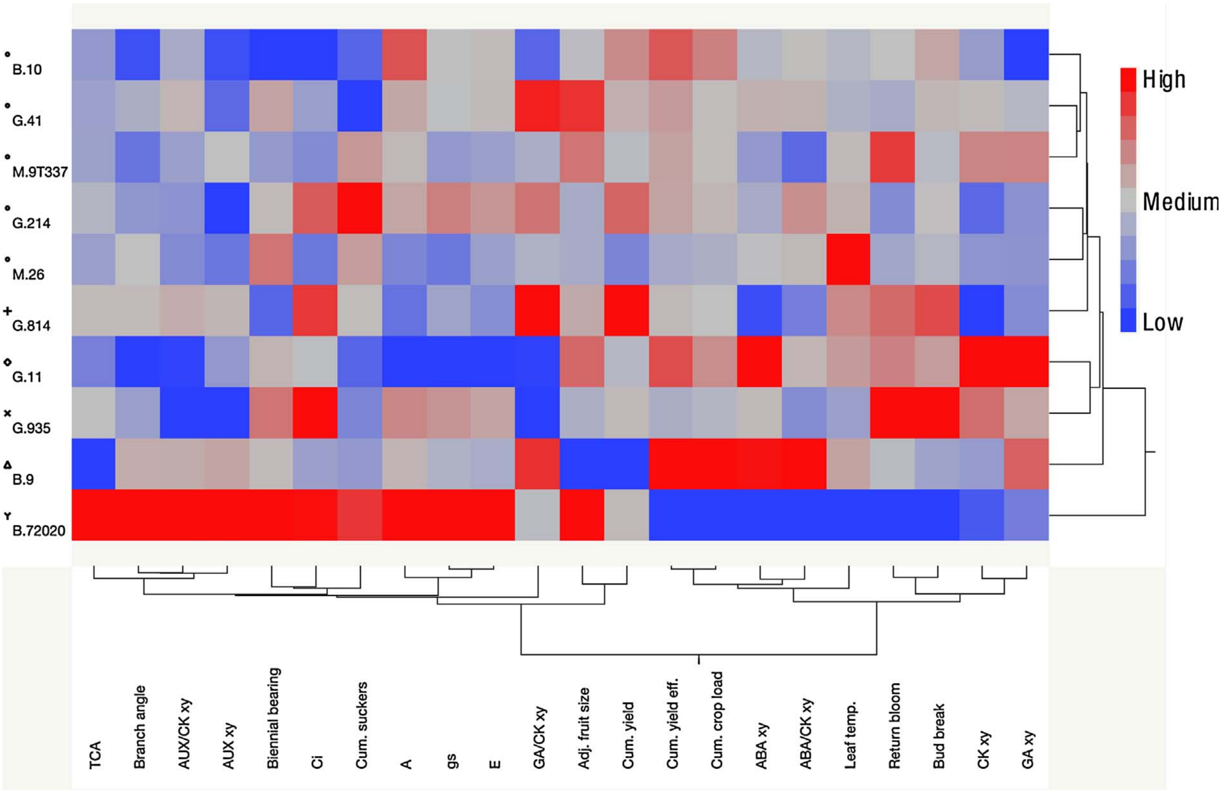


Fig. 3. Clustering of the 10 rootstocks based on their trunk cross sectional area (TCA) (cm^2), branch angle (°), auxins/cytokinins ratio in xylem (AUX/CK xy) (ng/g fresh weight), auxins (AUX) in xylem (ng/g fresh weight), mean biennial bearing index, CO_2 internal concentration (Ci) (ppm), cumulative number of suckers, carbon assimilation (A) (μmol CO2 m^{-2} leaf area s^{-1}), stomatal conductance (gs) (gs, mmol m^{-2} s^{-1}), transpiration (E) (mmol H2O m^{-2}leaf area s^{-1}), gibberellins/cytokinins ratio in xylem (GA/CK xy) (ng/g fresh weight), adjusted fruit size (g), cumulative yield (kg/tree), cumulative yield efficiency (kg/cm^2 TCA), cumulative crop load (fruit number/cm^2 TCA), ABA xy (ng/g fresh weight), ABA/CK xy (ng/g fresh weight), leaf temperature (°C), return bloom (%), bud break (%), CK xy (ng/g fresh weight) and GA xy (ng/g fresh weight).

for t-ZR, with the highest content on G.11 and the lowest on G.814 and G.214. The highest ABA/CK ratio was observed on B.9, whereas B.72020 had the lowest value. On the other hand, B.72020 had the highest AUX/CK ratio value; and G.11 and G.935 the lowest. No significant differences among rootstocks were observed regarding the GA/CK ratio.

Taking into account all the studied variables, rootstocks were clustered within six different groups (Fig. 3). In addition clustering the variable values revealed which variables are connected. TCA, branch angle, gas exchange variables, and biennial bearing were associated (correlated) with auxins and AUX/CK concentrations in xylem. On the other hand, yield efficiency, crop load, leaf temperature, return bloom and bud break were correlated with ABA, cytokinins (CK), and ABA/CK in xylem. B.10, G.41, M.9T337, G.214 and M.26 were clustered within the same group. G.814 was similar to the former group but had higher cumulative yields, lower ABA and ABA/CK content, and a more uniform bud break. G.11 was clustered alone, most likely due to its higher CK and ABA content and lower auxins and gas exchange rates. G.935 had also low auxins content but lower CK and ABA compared to G.11. In addition, G.935 also had higher return bloom and a more uniform bud break than G.11. B.9 and B.72020 were completely opposed to each other, while B.9 was the smallest stock of the trial with highest ABA content in xylem; B.72020 was the most vigorous with highest carbon assimilation, stomatal conductance, transpiration, and auxins content; whereas ABA and CKs were the lowest. Lower number of suckers was observed on G.41, G.11, B.10, and G.935.

Bud break was negatively correlated with auxins content in xylem, whereas no high correlations were observed for the cytokinin content (Fig. 4). Return bloom was negatively associated with auxins content in xylem, especially indole-3-acetic acid (IAA).

Branch angle and TCA were highly positively correlated with auxins and AUX/CK (Fig. 5). Fruit size was associated with high ABA/CK values. Yield efficiency, crop load and their cumulative were highly related with high ABA and ABA/CK content in xylem.

4. Discussion

M.26 was observed to be slightly smaller than M.9T337 in our trial. However, this stock has reported to be more vigorous, more similar to G.935 than M.9, when using the same weak growing cultivar (Robinson et al., 2011a), or with other cultivars such as ‘Fuji’, ‘McIntosh’ or ‘Gala’ (Autio et al., 2013; Autio et al., 2011). In addition, B.9, the most dwarfing stock of the trial, less vigorous than G.11 in our study, has reported to be more vigorous in previous trials, similarly to M.9T337 (Marini et al., 2006b; Robinson et al., 2011a; Robinson et al., 2011b). Growth and yield can be affected differently depending on the rootstock by the apple replant disease (ARD), a soil-borne complex of pathogens (mostly fungi) accumulated in the root-zone (Isutsa and Merwin, 2000; Mazzola, 1998; St Laurent et al., 2010). Our trial was set in a replanted orchard, and B.9 and M.26 are reported to be more sensitive to apple replant disease than most of the Geneva rootstocks, like for instance G.11, G.41, and G.935 (Kviklys et al., 2014; Robinson et al., 2003). Therefore, we may assume that disparity of tree sizes for B.9 and M.26 observed in this trial compared to previous studies might be explained by the ARD tolerance.

B.9, followed by G.11, B.10 and G.41 had the highest cumulative yield efficiencies. G.11 and B.10 were the stocks with the flattest branches, and B.72020 had significantly more upright branches and less yield efficiency. Branch angle was a key factor driving yield efficiency and crop load in our study. According to the observed negative correlation in the PCA analysis, the flatter the branches, the more efficiency we could expect in terms of productivity. Yield increase induced by

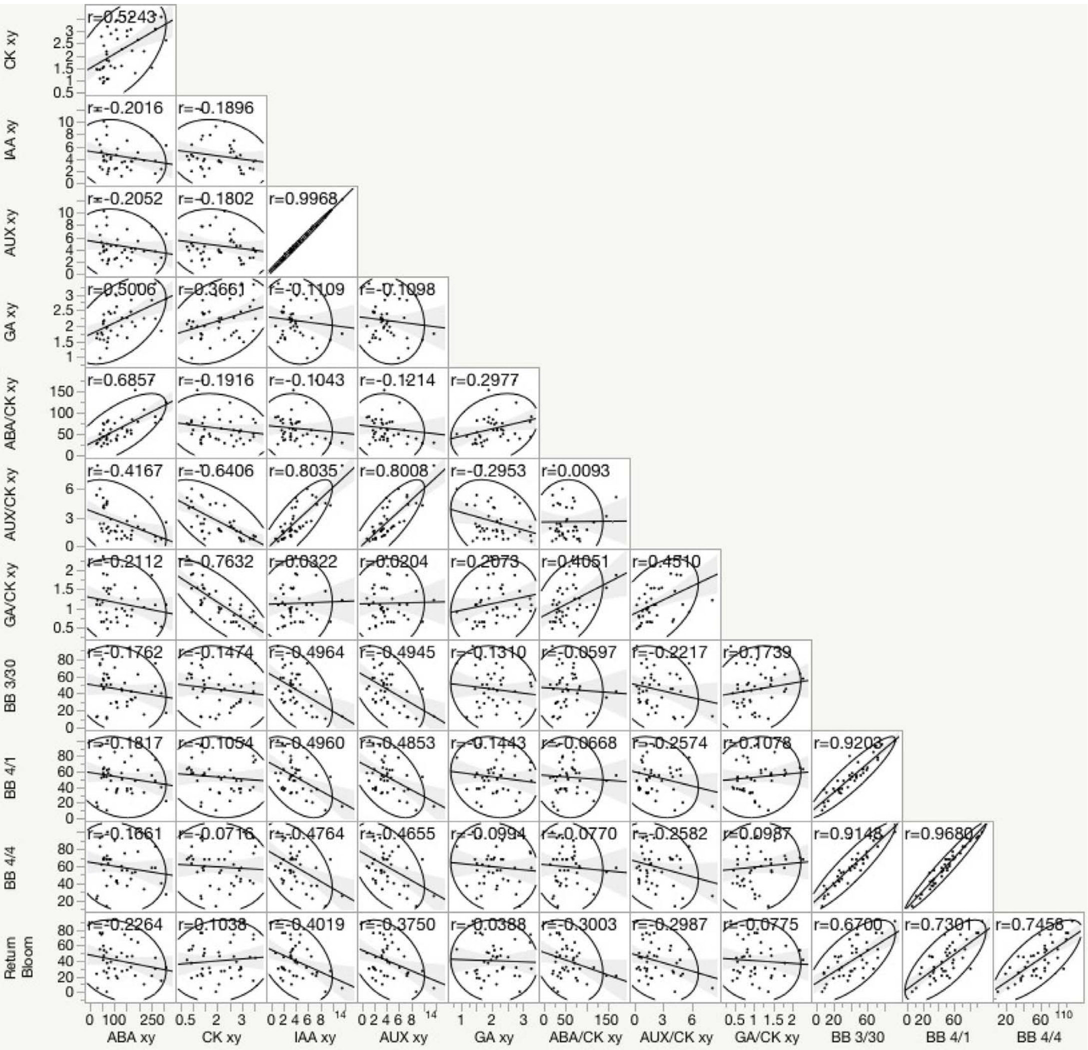


Fig. 4. Multivariate correlations among hormones in xylem (xy) of abscisic acid (ABA), cytokinins (CK), indole-3-acetic acid (IAA), auxins (AUX), gibberellins (GA), ABA and CK ratio (ABA/CK), auxins and cytokinins ratio (AUX/CK), bud break (BB) at three different dates (March 30th, April 1st and April 4th), and return bloom.

more horizontal branches has been previously described (Forshey and Elfving, 1989; Hollender and Dardick, 2015). Lauri and Lespinasse (2001) reported higher number of fruits and larger sizes when bending was applied. Furthermore, we observed differences on branch angle depending on the rootstock. Similar observations were made by Fazio and Robinson (2008) and Van Hooijdonk et al. (2011), when they reported that rootstock determined the number of feathers and their angle, modifying the scion architecture. Comparably, Kapłan (2010) observed how diverse rootstocks induced different number of shoots on the same scion. Following B.72020, B.9 was the next rootstock with higher branch angles in our study, and the least cumulative yield. In our trial, trees on B.9 had stunted growth, not filling the space and compromising yield. Since yield efficiency equals to yield per cm^2 of TCA, high yield efficiency values on B.9 could be explained most likely by their small TCA.

According to the PCA analysis, the higher the yields and the more the fruit number, the lower the return bloom. The effect of fruit number on return bloom has been widely accepted and documented, especially for biennial bearing cultivars (Harley et al., 1942; Jonkers, 1979; McLaughlin and Greene, 1991a). While less return bloom was observed on stocks that had higher yield in the previous year (B.72020), not all the stocks behaved the same way. For instance, G.814 and G.11 were among the stocks with higher yields, but also had higher return bloom in the following year. B.72020 had significantly much higher levels of auxins than G.814 and G.11. The role of auxins, and in particular indole-3-acetic acid (IAA), has been suggested to inhibit floral induction (Bangerth, 2009). In our study biennial bearing was not closely related to return bloom or productivity, suggesting that other variables could be driving it. Lespinasse and Lauri (1996) observed higher alternate bearing on more upright varieties. High biennial bearing indexes were observed in our study, with significant differences depending on the rootstock. Higher values were observed for B.72020, but since the second most vigorous rootstock G.814 had one of the lowest alternate bearing indexes and highest branch angles, we cannot relate biennial bearing with stock vigor or branch angle. A study made by Kviklys et al. (2016) in Lithuania did not find any significant differences regarding biennial bearing and rootstock; however no Geneva rootstocks were included in their study. In our study, M.9 was less biennial than their findings. In both studies, it was used scion cultivars that are prone to biennial bearing; therefore scion/rootstock interaction may also explain differences observed in both studies.

Similar to our experiment, a correlation between tree size and gas exchange rates has also been observed by Tworkoski and Fazio (2011), when they reported higher photosynthesis and transpiration rates on vigorous trees compared to dwarfing rootstocks. Looking at the PCA study, fruit size was highly correlated with leaf transpiration; the higher the transpiration the larger the fruit. ABA was previously associated in controlling abscission or bud dormancy, but recent studies suggested that its role is also related to stomatal closure in response to water stress as a mechanism to save water (Davies, 2004; Sauter et al., 2001;

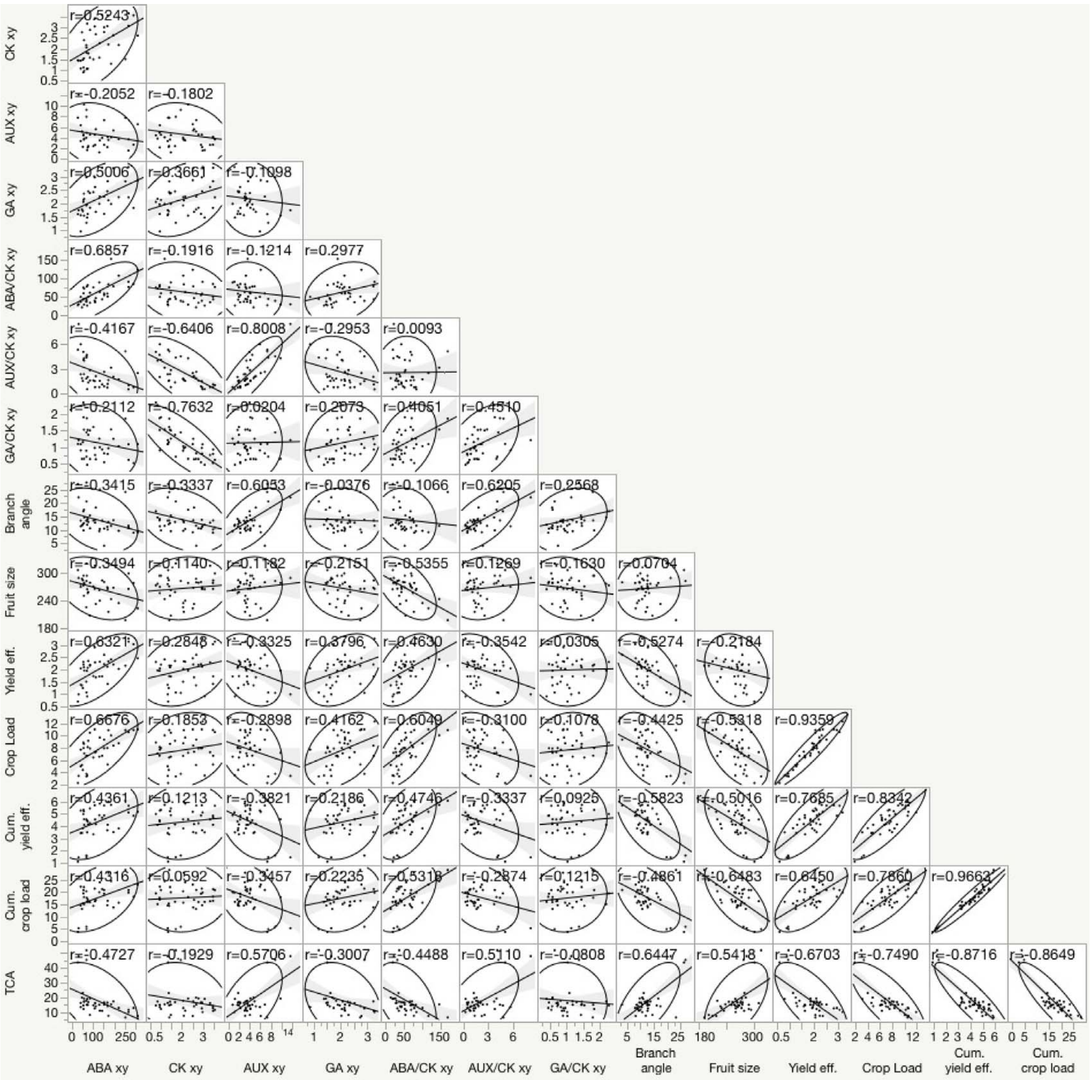


Fig. 5. Multivariate correlations among hormones in xylem (xy) of abscisic acid (ABA), cytokinins (CK), auxins (AUX), gibberellins (GA), ABA and CK ratio (ABA/CK), auxins and cytokinins ratio (AUX/CK), gibberellins and cytokinins ratio (GA/CK), branch angle, fruit size, yield efficiency, crop load, cumulative yield efficiency, cumulative crop load and trunk cross sectional area (TCA).

Zeevaart and Creelman, 1988). Hence, it might be assumed that stocks with higher ABA content will have less gas exchange due to stomatal closure, and fruit size may be affected as well. We observed high negative correlation between ABA − ABA/CK and fruit size and TCA. B.9 and G.11 had the highest ABA content in xylem, whereas B.72020 and G.814 had the lowest, confirming also the negative correlation between ABA and tree size. Tworkoski and Fazio (2011) suggested that the elevated ABA in dwarfing stocks might reduce hydraulic conductivity, and so affecting the tree size. In addition, smaller tree sizes and high ABA content have been proposed as more suitable for dry climates (Tworkoski and Fazio, 2015), and in some cases application of ABA and DL- β-aminobutyric acid (BABA) has been observed to confer dehydration protection in apple trees (Tworkoski et al., 2011). The use of rootstocks that confer higher ABA levels has been suggested to improve the water use efficiency and drought resistance in both apple and grapevines (Soar et al., 2006; Tworkoski et al., 2016). Since we observed important differences among rootstocks and their ABA content, we may suggest that G.11 and B.9 would be the most suitable rootstocks of the trial to be used under drought conditions.

We observed comparable bud break pattern among rootstocks that had similar cytokinin content. The role of cytokinins on bud break has been previously reported. For instance Jones (1973) promoted bud break of isolated apple shoots by applying xylem sap with high cytokinin levels that was previously extracted from the roots. Furthermore, Belding and Young (1989) reported the cytokinin *trans*-Zeatin riboside (t-ZR) to be necessary for bud break, but not as the initial causal agent. T-ZR has already been reported to be the main cytokinin in xylem (Davies, 2004). Coinciding with other authors (Cutting et al., 1991; Jones, 1973), the main cytokinin that we found in xylem was t-ZR. Significant differences were observed among rootstocks, G.11 had the highest levels, whereas B.72020 had the lowest. The sole influence of CKs on bud break was not clear in this study, though hormone analysis was done at full bloom. More uniform bud break was observed on stocks that showed high total CK levels, like G.11 and G.935. However, G.814 also had a very uniform bud break and it was one of the stocks with the lowest CK content. The fact that in our study a more uniform bud break pattern was not always associated with higher cytokinin content may indicate that not only cytokinins but also other variables may regulate bud break. For instance, bud break was also negatively correlated with auxins content in xylem. G.814 had considerably higher AUX:CK levels than G.11 and G.935. Some authors suggested that synthesis and levels of auxins and CK are reciprocally affected through hormonal signals (Aloni, 2001; Aloni et al., 2005). According to that, it is not just the CK and auxins content that might affect bud break and tree performance, but how the synthesis of one type hormone would affect production and translocation of other hormones.

In our study, G.11 and G.935 had the lowest AUX/CK rates, whereas B.72020, B.9, and G.814 had the highest. Tworkoski and Miller (2007) pointed to the AUX:CK ratio as the main factor regulating bud break, but also, and coinciding with our results, branch angle and TCA. The role of auxins on growth vigor has also been reported on *Prunus* rootstocks (Sorce et al., 2007; Sorce et al., 2006).

Gibberellins are mainly synthesized in shoots and seeds (Davies, 2004). However, coinciding with Motosugi et al. (1996), we found GAs in xylem, suggesting that they might also be synthesized in roots as well. The most abundant gibberellins found in our study were GA19, GA53, GA44 and GA24. Significant differences were observed only for GA53, with highest levels on B.9 and lowest on G.41. GA53 is reported to be biologically inactive, but is a precursor of the bioactive GA1 (Davies, 2004; Yamaguchi, 2008). GA1 has been described as the primarily gibberellin responsible for stem elongation (Davies, 2004), however, the role of gibberellins on tree performance was not clear in our study.

The performance trend we observed for the different rootstocks regarding vigor, yield and efficiencies needs to be confirmed during the coming years. However, yield efficiencies, branch angle, hormone profile and how these variables reciprocally interact are not likely to change in the future. The results of the current study provide useful information about how the main Malling, Budagovsky and Geneva rootstocks affect vigor, productivity and yield efficiency on a weak growing cultivar such as ‘Honeycrisp’. In addition, results prove how the hormone profile of the scion, bud break pattern and biennial bearing might also be affected by the rootstock. Findings provided by this study will be helpful suggesting the right rootstock to test for biennial bearing cultivars, hot dry climates, and for low chilling areas where irregular bud break may be a problem.

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