Chapter

Stock Influence on Growth, Morphological and Biochemical Leaf Parameters *Prunus domestica* L.

Svetlana Motyleva, Galina Upadysheva, Tatyana Tumaeva and Ivan Kulikov

Abstract

Vegetation growth peculiarities and morphological and physical-biochemical features of *Prunus domestica* L. Utro and Yaichnaya Sinyaya varieties leaves grafted on different growing strength rootstocks were studied. Low-growing clonal rootstocks 140–1 and Novinka decreased the trees growing strength on 15–20% in comparison with strong-growing stocks; medium-growing rootstocks OPA-15-2 and OP-23-23 reduced it on 10%. The longest growing activity and the largest sprouts length was stated on these rootstocks as well, i.e. 1.3–1.4 times more than on other ones. Stable sprouts average length decrease was registered on grafted stocks 140–1 and Novinka. Leaf surface index value on the trees grafted on clonal rootstocks OPA-15-2 and OP-23-23 was on 40% higher than on control, i.e. 4.3 leaves m2/crown projection area m2. Optimal values of total increment, sprouts average length, leaves area and the largest part of physiological-biochemical parameters were stated at medium-growing clonal rootstocks OPA-15-2 and OP-23-23 use. Plum leaves blades were hypostomatic; numerous stomata were located on the abaxial (bottom) side of leaves. Stomata were located in interveinal space irregularly. Stomata length size varied from 14.6 μm (Utro/seedlings) to 22.1 μm (Yaichnaya Sinyaya/OP-23-23). The rootstock has influence on the process of photosynthesis, antioxidant activity, accumulation of minerals and metabolic answerin the leaves.

Keywords: *Prunus domestica* L., scion-stock combinations, growth and development, leaf, scanning electronic microscopy, physiological parameters

1. Introduction

The popularity of domestic plum (*Prunus domestica* L.) in different horticultural zones of Russia is connected with ecological plasticity, winter resistance, early maturity and stable productivity of cultivated varieties [1, 2]. New clonal stocks are used to duplicate new valuable varieties of fruit crops and to create intensive plantations because they favorably influence adaptivity, early maturity and productivity of grafted plants [3, 4]. The spread, the start of fruiting and the productivity of grafted plants depends on the rootstock. Seedling rootstocks (seedlings of *Prunus domestica* L. and *Prunus cerasifera* Ehrh.) and clonal ones of different growing spread, i.e. strong-growing – 13-113, medium-growing – OPA-15-2, OP-23-23,

SVG-11-19 and low-growing – 140-1, Novinka, VVA-1 are used for plums cultivation in the Central region of Russia [5].

Many questions devoted to plum varieties propagation and scion-stock combination selection are successfully solved in the world; at the same time, the life length of grafted plants and the harvest quality depending on a variety and a rootstock are not studied enough. The major part of the researches in this branch of study were held by [6–8], who found out that photosynthesis intensity varies depending on a variety and scion-stock combination. The influence of rootstock on the quality of plum fruits is shown in the works [9, 10].

This present study was planned to analyze the least studied morphological and biochemical characteristics, i.e. – growing activity and the sprouts length, lea fsurface index on the trees, Stock influence on leaf morphological features and parameters, on photosynthetic pigments synthesis, on antioxidant activity and phenol compounds sum accumulation in plum leaves, on ash composition and on *Prunus domestica* L. leaves metabolic answer.

2. Studies results

2.1 Studies place, objects and methods

The field researches were held in 2018–2020 on the experimental *Prunus domestica* L. plantations, located at laboratory plot of Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery (FHR CBAN) in Moscow region (55° 56′ of North latitude, 37° 64′ East longitude). The garden was planted in 2010. The plantation overall area was 0.5 ha. The garden of intensive type was set out using the scheme 5 x 2.5 m. The soil in the row spacing was agrogenic (**Figure 1**).

The leaves of *Prunus domestica* L. Utro and Yaichnaya Sinyaya varieties on 5 stocks, i.e. seedlings *Prunus domestica* L., Novinka (*Prunus bessyi* L.H. Bailey x *Prunus ussuriensis* Kovalev&Kostina), OP-23-23 (*Prunus pumila* L. x *Prunus salicina* L. x *Prunus persica* Stokes), OPA-15-2 (*Prunus pumila* L. x *Prunus salicina* L. x

Figure 1.
Plum plantations: 1-blossoming garden, row spacing – Agrogenic soil.

Prunus cerasifera Ehrh.) and 140–1 (*Prunus bessyi* L.H. Bailey x *Aflatunia ilmifolia*) were the object of the scientific studies, as well as the understudies rootstocks leaves. The leaves samples were taken from the medium part of the formed one-year-old sprouts (the beginning – mid July).

The biochemical researches were held in the Laboratory of Biochemistry and Physiology of FHR CBAN.

The understudied parameters included field registration (the trees growth, the crown volume, the total sprouts increment, leaf surface area) and the leaves laboratory studies (comparative micromorphology of the leaf adaxial and abaxial sides, stomata number and size, photosynthetic pigments content, antioxidant activity, phenolic compounds sum, ash composition and quality content of the leaves main metabolites). The leaves microsculpture and ash composition were determined on analytical REM JEOL JSM – 6010 LA (JEOL Ltd., Japan). Photosynthetic pigments Chl a and b and total carotenoids (Car) were studied on spectrophotometer Helios Υ UV–vis (USA) in accordance with the method [11], total phenolic amount was determined with Folin–Ciocalteu reagent in accordance with the method [12] and total antioxidant capacity the scavenging activity for the 2,2-dipheny l-1-picrylhydrazyl (DPPH) radical was determined in accordance with the method [13].

Metabolites quality composition, contained in plum leaf extracts was analyzed on JEOL JMS-Q1050GC (JEOL Ltd., Japan) via the method of gas chromate-mass-spectrometry in accordance with the method [14].

2.2 Stock influence on growth parameters

Stock influence on trees growth parameters were studied using tree height, crown volume, one-year-old sprout length, leaf surface area. Utro variety 8-year-old tree height was within the range of 2.8 m (140–1) – 3.2 m (seedlings). Yaichnaya Sinyaya variety height differences determined by stock were 0.6 m, and height varied from 3.1.m (140–01) to 3.7 m (seedlings). In comparison with strong-growing seedlings low-growing rootstocks 140–1 and Novinka reduced tree growing strength on 15–20%, medium-growing stocks OPA-15-2 and OP-23-23 – on 10%.

Depending on a rootstock, plum trees crown volume was within the range of 11.5 m3 (Yaichnaya Sinyaya/140–1) – 14 m3 (Yaichnaya Sinyaya/OP-23-23). Utro variety crown volume varies insignificantly on different rootstocks (12–13 m3).

Plum trees growing process intensity are characterized by active sprout growth duration and total one-year-old sprouts length. Domesticated plum active growth duration was from 32 days (Utro variety) to 40 days (Yaichnaya Sinyaya variety), and depending on a rootstock the fluctuations were 8–11 days. The longest active growth duration was registered on 140–1 and OP-23-23 rootstocks.

Average sprouts length that characterizes tree general state was within wide ranges, i.e. from 9.0 cm (Utro/Novinka) to 22.8 cm (Yaichnay Sinyaya/OP-23-23) and was 25.0 cm at average. At Yaichnaya Sinyaya variety this parameter was stable depending on a rootstock (18–22 cm) and 1.5 times higher than at Utro variety (**Figure 2**).

Stable reduction of average sprouts length was stated on grafted 140–1 and Novinka rootstocks. On OPA-15-2 and OP-23-23 rootstocks this parameter was 1.3–1.4 times higher (**Figure 3**).

The main indicator of growing processes activity is total sprouts length. This parameter was significantly less at the trees grafted on clonal rootstocks, i.e. 140–1 (110 m) and Novinka (120 m). On medium-growing OPA-15-2 and OP-23-23 sprouts buildup was 170–180 m and 1.2 times bigger than on seedlings.

The main indicator that characterizes plum tree crown leaf coverage level is leaf surface area. The size of the leaves varied significantly depending on the scion-rootstock combination (**Figure 3**).

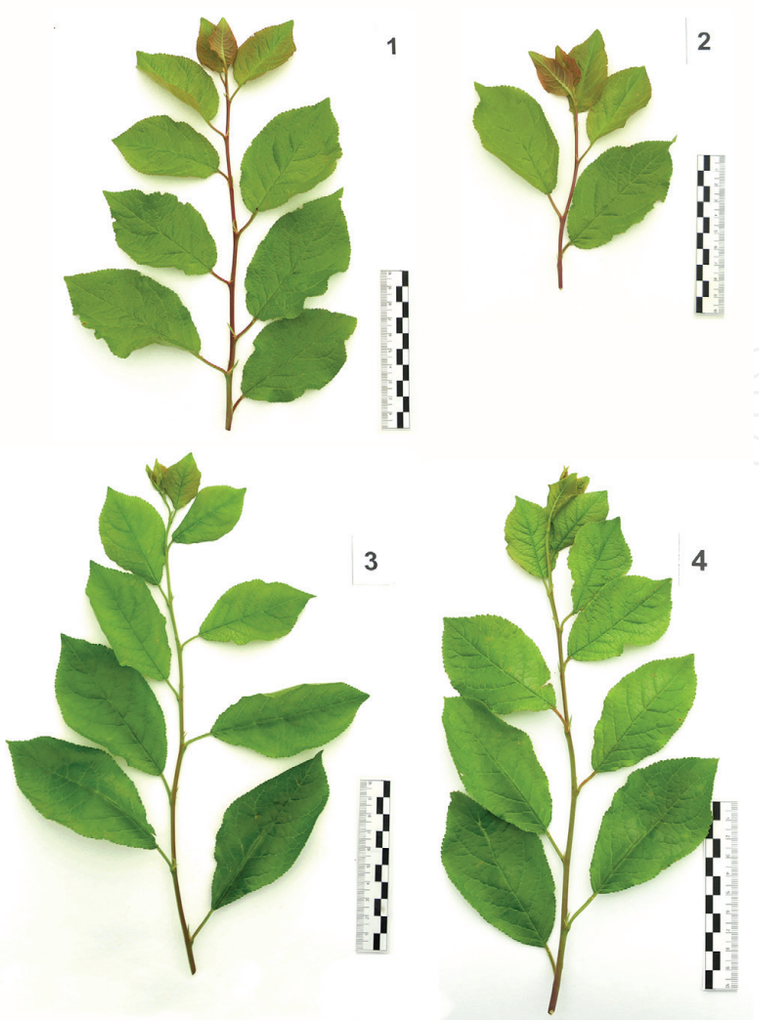

Figure 2.
Length of Prunus domestica *L. shoots depending on the rootstock.*

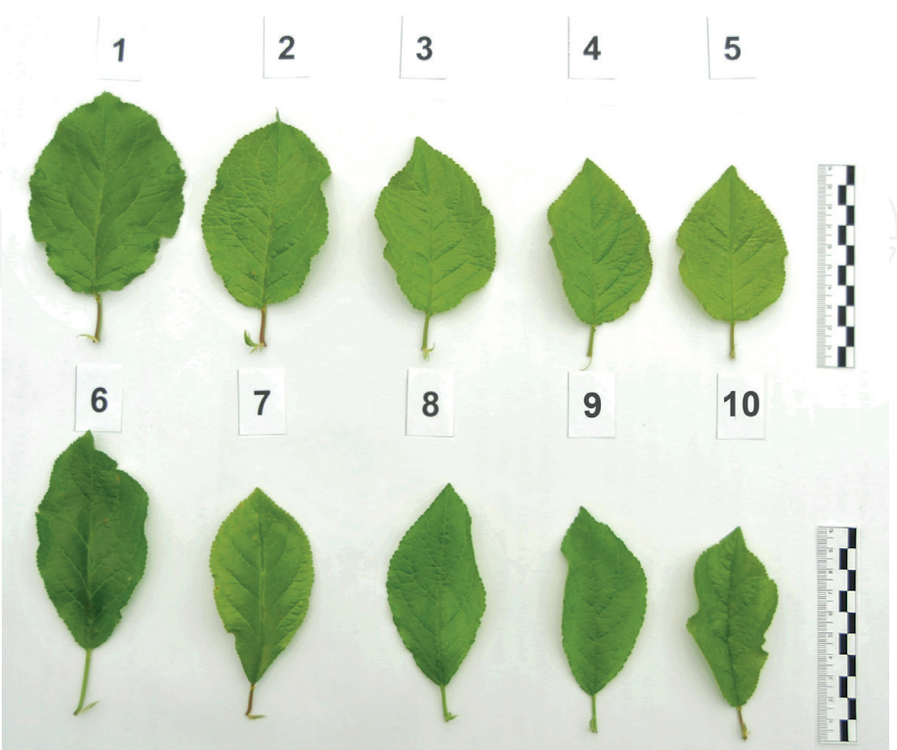

Figure 3.
The size of the leaves of Prunus domestica *L. depending on the rootstock.*

Leaves area was within the range of 21.4 m^2/tr. (Yaichnaya Sinyaya/seedlings) – 36.2 m2/tr. (Utro/OPA-15-2). At Utro variety the differences between low and strong-growing rootstocks were 9–11 m2/tr., and at Yaichnaya Sinyaya variety – 6-8 m^2/tr. More higher values at that were closer to physiological optimum. i.e. more than 30 m2/tr., were registered on grafted OPA-15-2 and OP-23-23 rootstocks. Less assimilation apparatus was formed on low-growing 140–1 rootstock than on control.

Plum trees leaf surface index depending on a combination was within the range of 4.3–7.2 leaves m2/crown projection area m2. Leaf surface index maximum values, i.e. 7.2 leaves m2/crown projection area m2, were registered at scion-stock combinations Utro/OPA-15-2 and Yauchnaya Sinyaya/OP-23-23. Leaf surface index value was on 40% at the trees grafted on OPA-15-2 and OP-23-23 rootstocks than on control, i.e. 4.3 leaves m2/crown projection area m2.

The main indicators of domesticated plum trees vegetative productivity, i.e. crown volume, total sprouts buildup, leaf surface area, depended not only on a variety, but also on a rootstock. Optimal values of total buildup, average sprouts length, leaves area were registered on medium-growing clonal OPA-15-2 and OP–23–23 rootstocks.

2.3 Stock influence on leaf morphological features and parameters

Morphological differences of various varieties leaves were studied using scanning electron microscopy (SEM). Adaxial epidermis consists of thick cells layer. Their surface is covered with firm cuticle with numerous folds or in the form of long stripes and colpi, microsculptural differences are presented in **Figure 4**. Plum leaves blades were hypostomatic; numerous stomata were located on the abaxial (bottom) side of leaves (**Figure 5**). Grafted plants leaves, as a rule, had well-developed rollers around stomata. Stomata were located in interveinal space irregularly. Stomata length size varied from 16.42 μm (Utro/seedlings) to 22.11 μm (Yaichnaya Sinyaya/OP-23-23). It is typical that the ratio of stomata length to their width, at variety-stock combinations was larger than at a rootstock. Such regularity was stated for all variety-stock combinations. Stomata density varied significantly from 436 ± 9

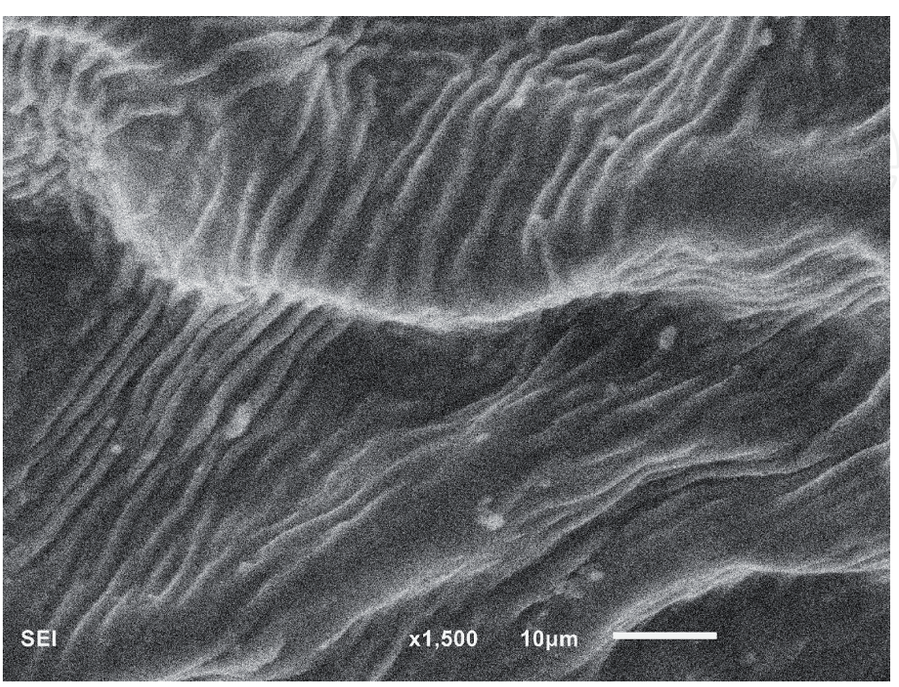

Figure 4.
Prunus domestica *L. leaf adaxial side.*

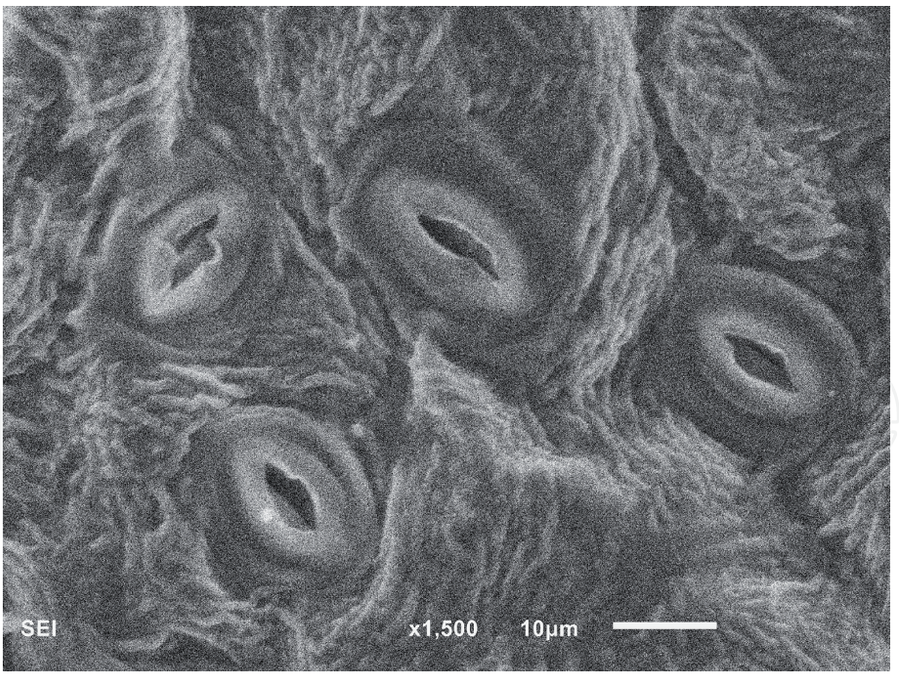

Figure 5.
Prunus domestica *L. leaf abaxial side.*

(Yaichnaya Sinyaya/OP-23-23) to 1000 ± 17 (Utro/seedlings) stomata on mm^2 at. Variation of mean indicators that characterize stomata and the density of stomata location on leaf surface are shown in **Table 1** using the example of the Utro variety.

2.4 Stock influence on photosynthetic pigments synthesis

Chlorophylls Chl a and Chl b content is one of the main indicators of domesticated plum trees vegetative productivity. Chl a content is 3–4 times higher than Chl b one at average and varies from 4.12 mg/ml (seedlings) to 13.71 mg/ml (OP-23-23). Chl a + Chl b sum content increase and the highest ratio Chl a/b were registered in Yaichnaya Sinyaya and Utro leaves on OPA-15-2 rootstocks and OP-23-23, that lead to higher intensity of photosynthesis process (**Figure 6**). The highest carotenoids content was registered in OP-23-23 rootstock leaf extracts (1.26 mg/ml), Utro/Novinka (0.9 mg/ml), Utro/OP-23-23 (0.79 mg/ml) and Yaichnaya Sinyaya (0.75 mg/ml) combinations (**Figure 7**). Consequently, photosynthetic pigments synthesis depended not only on a genotype, but also on a used rootstock.

2.5 Stock influence on antioxidant activity and phenol compounds sum accumulation in plum leaves

The capacity of plum leaf extracts to scavenge DPPH+ free radicals, which has been used as a measure of total antioxidant capacity (AA), and total phenolic

Stick-stok kombination	Number of stomata/mm^2	Stomata length	Stomata width	Stomatal index
Utro/seedlings	1000 ± 17	17.59 ± 0.14	9.57 ± 1.11	1.8
Utro/Novinka	800 ± 13	19.22 ± 1.24	11.23 ± 1.11	1.7
Utro/140–1	689 ± 21	16.42 ± 2.11	8.32 ± 1.14	1.9
Utro/OP–23–23	556 ± 22	18.26 ± 2.11	8.94 ± 0.85	2.0

Table 1.
Variation of mean indicators that characterize stomata and the density of stomata location on leaf surface.

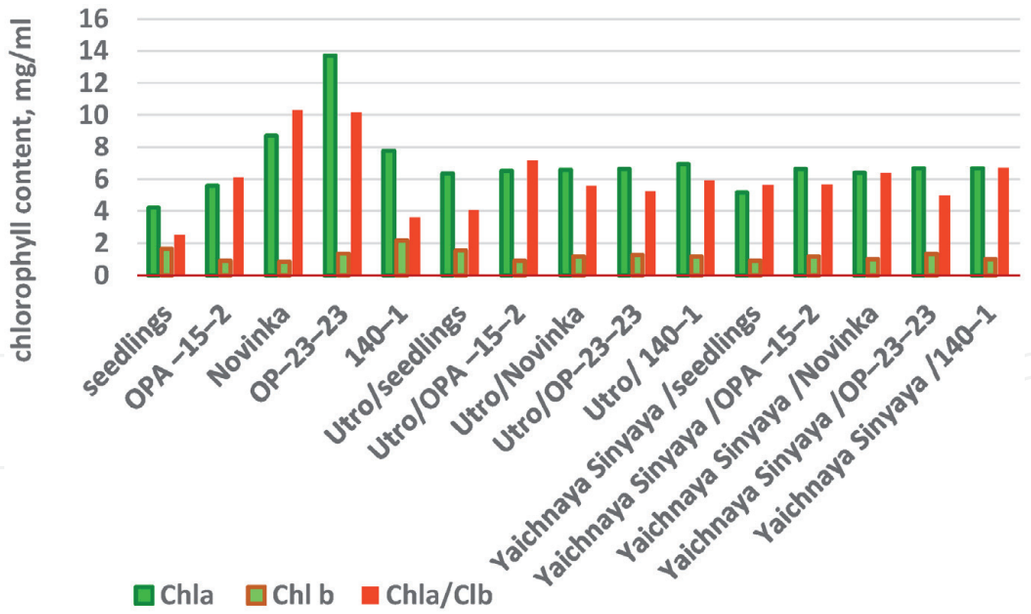

Figure 6.
Chlorophylls Chl a and Chl b and ratio Chl a/Chl content in the leaves of Prunus domestica rootstocks and scion-stock combinations.

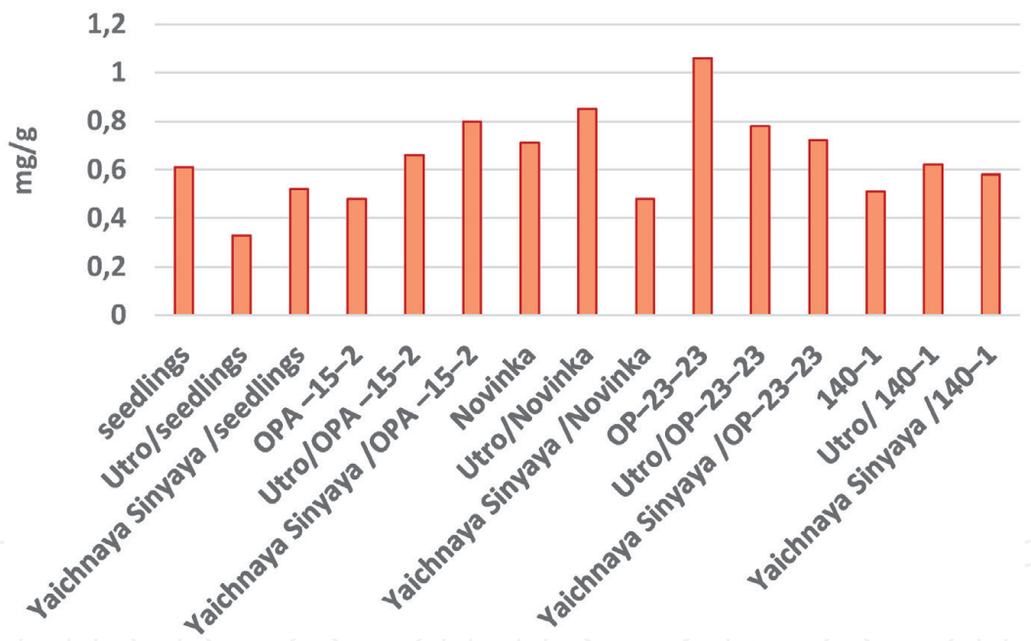

Figure 7.
Carotenoids content in the leaves of Prunus domestica *rootstocks and scion-stock combinations.*

content (TPC) are shown in **Table 2**. AA speaks about the presence of biologically active substances-antioxidants that are synthesized in plum leaves. Average AAA and AAM of ethanol extracts was 88.7%; average AA of water extracts was 73.8%. The maximum AAA (90.72%) and AAM (92.12%)values were registered in the leaves of Yaichnaya Sinyaya/Novinka combination; the minimal values were stated in the leaves of strong-growing *P. domestica* L. seedlings AAA (88.14%). Among the rootstocks the maximum AA values were marked in the leaves of Novinla and OP–23–23. The leaves of scion-stock combinations on these rootstocks showed high AAA and AAM values in comparison with other combinations. Consequently, a rootstock has influence on the synthesis of substances-antioxidants in the scion leaves. TPC in all the scion-stock combinations leaves is higher than in rootstocks leaves on 3–12 mg/g. The comparison of ethanol leaf extracts spectra of rootstocks

Samples	Determined indicators		
	AAA	AAM	TPS
Strong-growing *P. domestica* L. seedlings	88.14 ± 1.14	57.08 ± 1.11	13.68 ± 0.24
Yaichnaya Sinyaya/strong-growing *P. domestica* L. seedlings	89.76 ± 2.01	68.11 ± 1.21	16.48 ± 0.31
OPA–15–2	89.71 ± 1.14	79.15 ± 1.08	6.23 ± 0.21
Yaichnaya Sinyaya/OPA–15–2	88.55 ± 2.11	47.61 ± 0.89	11.42 ± 0.22
Novinka	90.72 ± 1.46	82.38 ± 1.24	9.83 ± 0.11
Yaichnaya Sinyaya/Novinka	88.51 ± 1.34	79.19 ± 2.01	14.63 ± 0.21
OP–23–23	90.55 ± 1.51	75.77 ± 2.12	9.19 ± 0.31
Yaichnaya Sinyaya/OP–23–23	90.72 ± 1.34	92.12 ± 2.13	14.51 ± 0.42
140–1	89.71 ± 1.24	79.31 ± 1.06	14.93 ± 0.61
Yaichnaya Sinyaya/140–1	89.36 ± 1.40	77.35 ± 1.42	23.48 ± 0.42

Table 2.
The antioxidant activity of water (AAA) and methanol (AAM) extracts, expressed in % and the total content of polyphenols (TPC), expressed in mg equivalent of gallic acid (mg/g TW) in the leaves of Prunus domestica *L.*

and scion-stock combinations was fulfilled in the range of 350–700 nm. The spectra profiles of Yaichnaya Sinyaya/seedlings, Yaichnaya Sinyaya/Novinka and Yaichnaya Sinyaya/OP-23-23 combinations are lower in the understudied wavelength range than the rootstocks profiles. The leaf extracts spectrum of Yaichnaya Sinyaya/OPA-15-2 combination in 200–350 nm range significantly varies from OPA-15-2 rootstock profile that speaks about absorbent substances presence in this spectrum region (**Figure 8**).

2.6 Stock influence on plum leaves ash composition

12 ash elements, i.e. P, S, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, were studied. The decreasing row of the elements content in the plum leaves ash is the following: K > Ca > Co > Mg > P ≈ S > Cu ≈ Zn > Fe > Mo > Cr ≈ Ni.

The principal proportion of the leaves ash composition was K, which was accumulated up to 25 mass % and Ca up to 10 mass % in ash (**Figure 9**). The maximum content of K is noted in the leaves of Yaichnaya Sinyaya and Novinka on OP-23-23

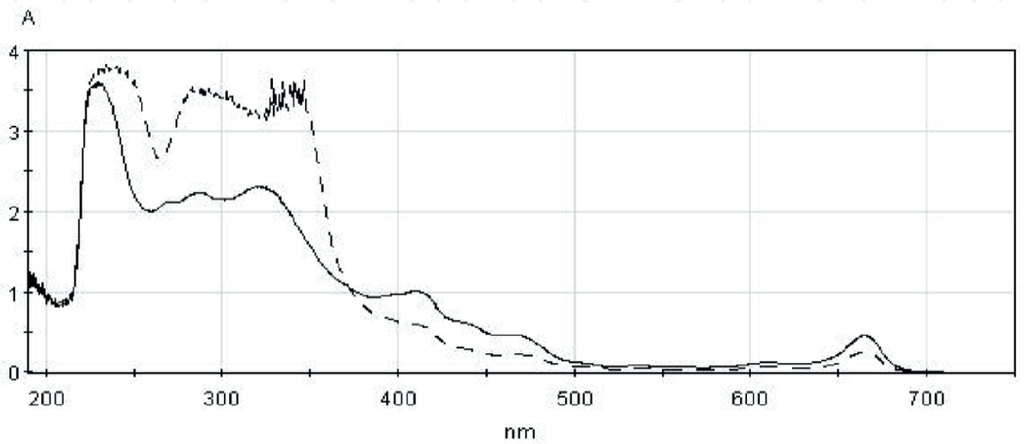

Figure 8.
Comparative spectra of Prunus domestica seedlings and Yaichnaya Sinyaya scion-stock combinations leaf extracts (____ - OPA–15–2; - - - - - Yaichnaya Sinyaya/OPA–15–2).

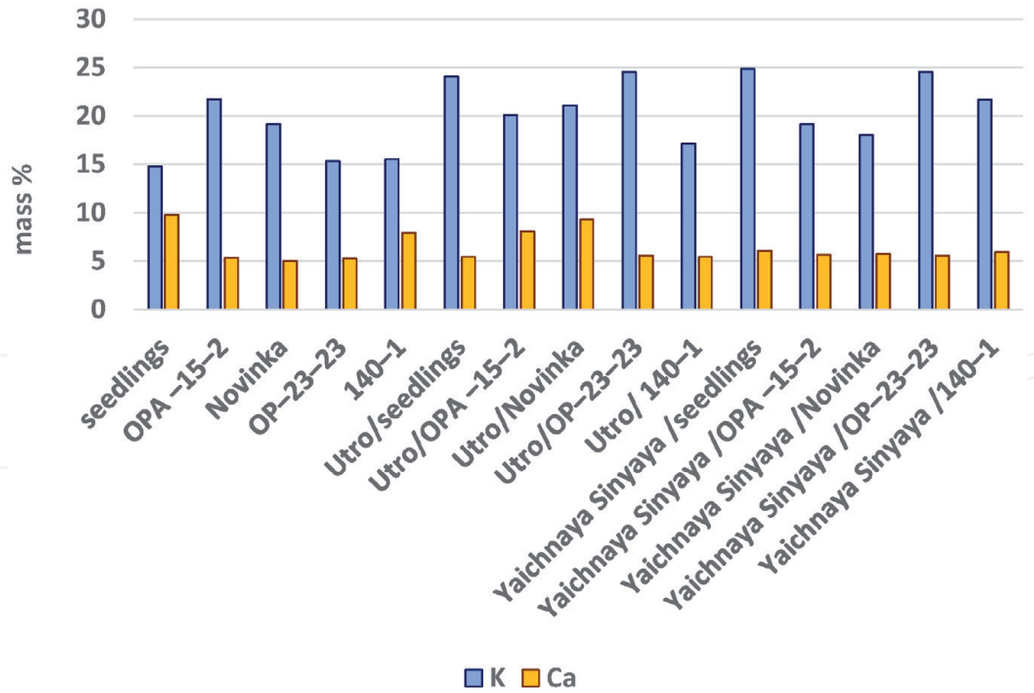

Figure 9.
The content of K and Ca in the leaves Prunus domestica *L.*

combination; the minimal values were stated in the leaves of strong-growing *P. domestica* L. seedlings, OP-23-23 and 140–1. The scientific literature shows that K is very mobile in the plants, and it is known that K plays the role of osmotic agent in the stomata opening and closing processes. Ca is found in cell walls in the form of calcium pectate which affects cell walls elasticity. The important role of these ash elements was also noted in plants adaptive processes [15, 16]. Co is involved in the processes of nitrogen uptake by plants. The maximum content of Co is noted in the leaves of Yaichnaya Sinyaya on of strong – growing *P. domestica* L. combination and Novinka; the minimal values were stated in the leaves of Utro on of OP-23-23 and Novinka combination. Mn is a part of the chlorophyll molecule and is involved

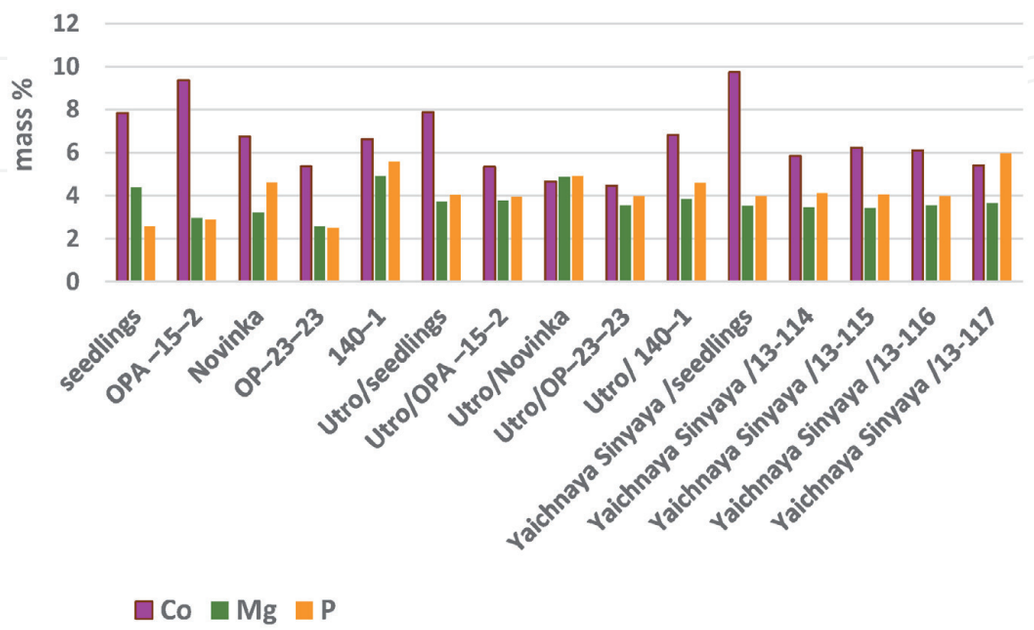

Figure 10.
The content of Co, Mg and P in the leaves Prunus domestica *L.*

in a number of enzyme systems. P is found in phospholipids and nucleoproteins; macro-energy connections between phosphate groups and serves as the main energy transfer agent in plants. The content of Mg and P ranges from 2.3 mass % (OP–23–23) to 4.8–5.8 mass % (140–1), **Figure 10**. The highest content of Cu, Zn and Fe in plum leaves was noted in the combination Utro/140–1 (**Figure 11**).

The certain oligo-elements Mo and Ni weare contained with in the range of from 1.2 (140–1) to 3.2 (Utro/OP–23–23) mass % – Cu and Ni from 2.1 (Yaichnaya Sinyaya/140–1) to 5.6 (Utro/140–1) mass % (**Figure 12**).

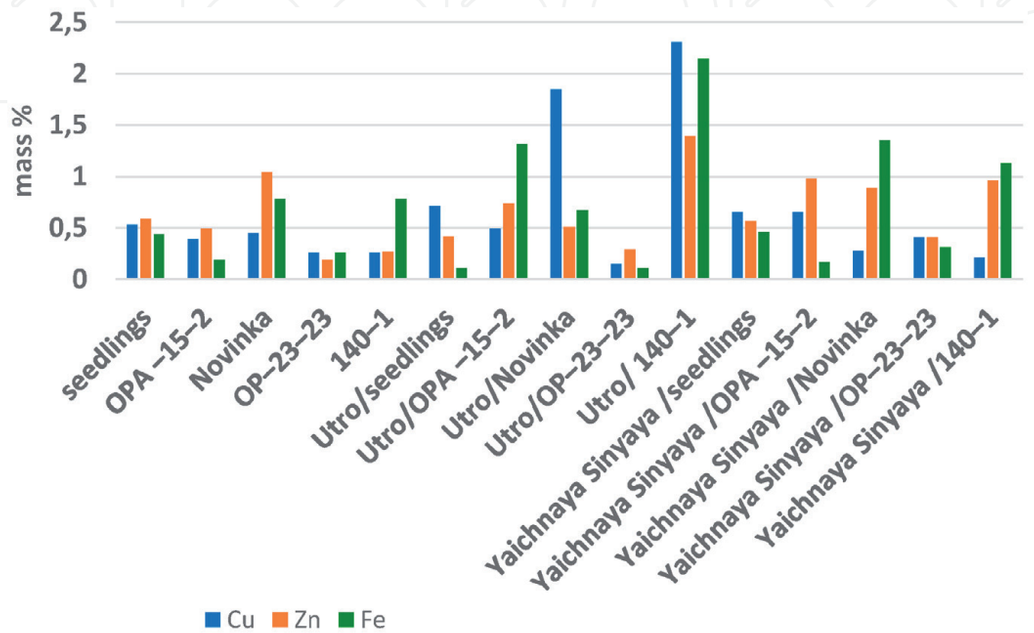

Figure 11.
The content of Cu, Zn and Fe in the leaves Prunus domestica *L.*

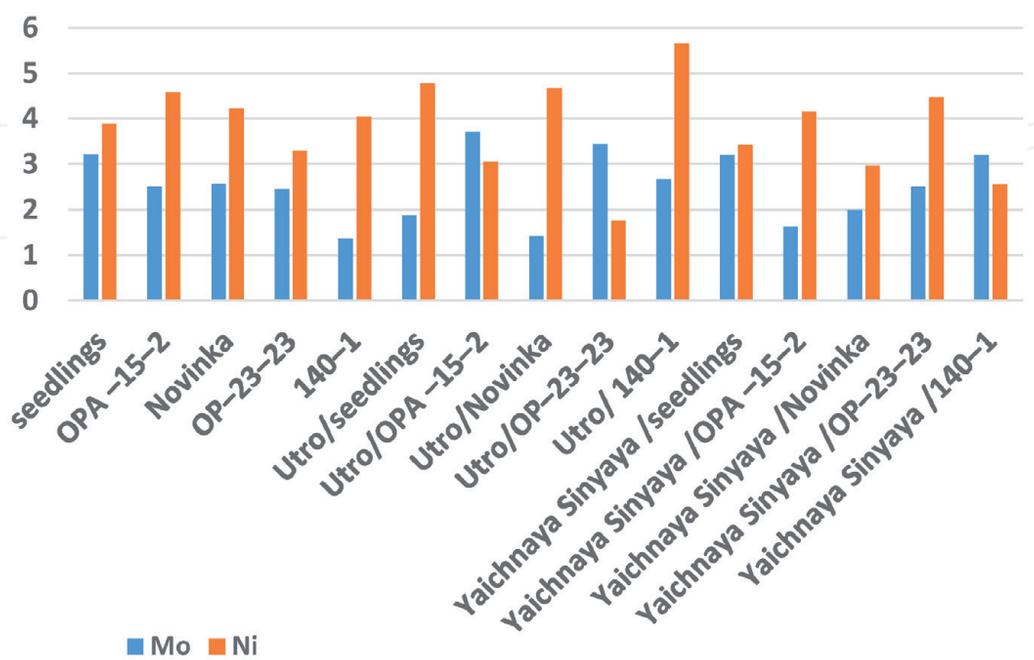

Figure 12.
The content of Mo and Ni in the leaves Prunus domestica *L.*

2.7 Stock influence on plum leaves metabolic answer

Metabolites component composition of rootstocks and scion-stock combinations leaves was fulfilled using the method of gas chromate-mass-spectrometry. Comparative chromatogram of Yaichnaya Sinyaya/seedlings combination with other combinations are given in **Figure 13**. In the whole, chromatographic profiles are alike and are characteristic for *Prunus domestica*. The main differences may be found in quantitative content of the substances that are identified by the peaks with the following retention time: 13.50 min (Erythronic acid), 13.43 min (glycerol), 14.27 min (Hellenic acid), 16.37 min (Lactic acid), 19.53 min (levoglucosan), 21.02 min

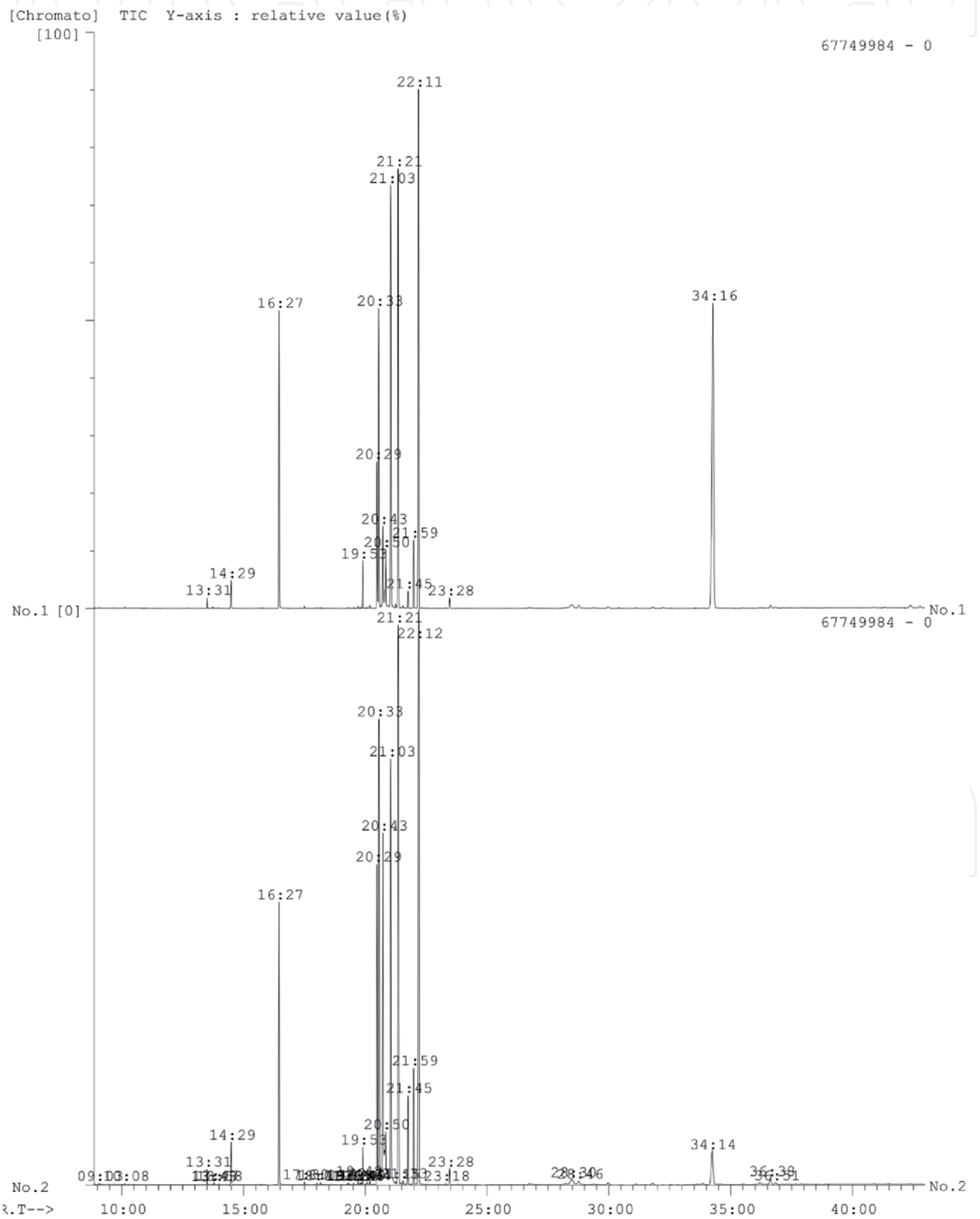

Figure 13.
Comparative chromatograms of Novinka rootstock (above) and Yaichnaya Sinyaya/Novinka (below) combination leaf ethanol extracts.

(Quinnic acid) and 23.27 min (antioxidant mio-inositol). The highest peak of mio-inositol and its content respectively were registered in the leaf extracts of Yaichnaya Sinyaya/OPA-15-2 combination). Our studies showed that in the leaves of Yaichnaya Sinyaya variety on OP-23-23 rootstock Quinnic acid and Chlorogenic acid that play a great role in adaptive processes [16] are synthesized on 15% and 10% more respectively than in a rootstock leaves. Sucrose, Fructofuranose and Fructose content in Yaichnaya Sinyaya variety leaves was 2.5–3 times more than in a rootstock leaves.

3. Conclusions

Understudied *Prunus domestica* varieties demonstrated different growth character and morphological-biochemical peculiarities depending on the used rootstock. While analyzing the data, it was stated that growth parameters, photosynthetic processes and metabolites synthesis in plum leaves differed in similar experimental environmental conditions. They depended on a variety and a rootstock inside the variety. Both the variety and the rootstock have special influence on photosynthesis process intensity, moreover the received results varied from one combination to another one. For example, Yaichnaya Sinyaya variety on OPA-15-2 and OP-23-23 rootstocks were the highest and showed the largest sprout formation. These differences have influence on plum trees light regime that causes the changes in morphology and leaves chemical composition (photosynthetic pigments and metabolites synthesis) and mineral substances accumulation.

Such information is useful for the grafted plants physiology evaluation and scion-stock combination choice.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

None.

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