



PLUM (*PRUNUS ROSSICA* EREM.) FRUIT FIELD AND LABORATORY RESEARCHES DEPENDING ON THE SCION-STOCK COMBINATIONS

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ABSTRACT

The results of the field observations and biochemical researches of plum (*Prunus rossica* Erem.) fruits grown in the garden of intensive type with agrogenic soil in row spacing are presented in the article. The fruits of plum breeds Kubanskaya kometa and Naidena were gathered from the trees grafted on different stocks. The influence of the stock on the trees productivity, the fruit weight and biochemical structure of the plum fruits were studied. Basic chemical analyses, ascorbic acid concentration, antioxidant activity and fruit ash content were determined. The variation limits of the parameters under study depending on the used stock were shown. The best degustation evaluation parameters were found at the combinations of Kubanskaya kometa on stocks OPA-15-2 and Novinka, Naidena on stocks OPA-15-2 (more than 4,5 points). The high positive correlation ($r = 0.92$) was marked between soluble solids and sugars content. The researches about the stock influence on the plum fruits titratable acidity change we have not found. The correlation between AA and ascorbic acid content in the fruits was determined ($r = 0.7$). The decreasing row of the elements content in the plum fruits ash is the following: $K > P > Mg > Ca > Mo > Cu > S > Ni > Fe \approx Si > Mn$. The proportion of K is from 18.67 (Naidena/OPA-15-2) to 27.48 (Kubanskaya kometa/Novinka); P from 3.35 (Kubanskaya kometa/seedlings *P. cerasifera* Ehrh. to 6.39 (Kubanskaya kometa/Novinka), Mg from 1.11 (Naidena/OPA-15-2) to 2.43 (Naidena/seedlings *P. cerasifera* Ehrh.) and Ca from 0.39 (Naidena/13-113) to 1.85 (Naidena/OPA-15-2) mass % respectively. The proportion of the rest elements is not more than 1 mass %. Under the OPA-15-2 stock influence the trees productivity and the single fruit weight increased, soluble solids, sugars and ascorbic acid concentration in the fruit in comparison with standard stock by the seedling rootstock of alycha (*Prunus cerasifera* Ehrh. seedlings). While ingrafting by Novinka stocks the parameters under study decreased.

Keywords: plum; clonal stock; scion-stock combinations; biochemical structure of fruit

INTRODUCTION

One of the most perspective plum breeds cultivated on the European territory of Russia is plum Russkaya (*Prunus rossica* Erem.). The first breeds were received by the member of the Academy of Sciences G. V. Petrov on Krymskaya EBS ARIR (Krandodarskiy Krai) as the result of cross-species hybridization of breed Skoroplodnaya (*Prunus ussuriensis* L.) and alycha breed Pionerka (*Prunus cerasifera* Ehrh.) (Eremin, 2003). At the present time thanks to winter-hardy breeds *Prunus rossica* Erem. they are cultivated not only in the southern areas, but in the Central region of Russia (Upadysheva, 2017). It is appreciated for the high eating qualities of the fruit, their curative and dietic properties (Motyleva et al., 2017). Plum fruit are rich in organic mineral substances: mono- and disaccharides, pectin, vitamins, flavonoids and other

biologically active substances (Kukurová et al., 2015; Kulichová et al., 2016).

The researches of the totally different fruit antioxidant ability measurements showed that plums have one of the highest antioxidant activities (Imeh and Khokhar, 2002). Among the molecules that show antioxidant activity in plums it was discovered that the phenolic compounds contribution is essentially higher than vitamin C and carotenoids contribution (Gil et al., 2002). For the plum reproduction and the intensive gardens creation the clonal stocks are used (Dekena et al., 2017). As our experiments with sweet cherry show the stock influences the grafted plants development and metabolism determining the harvest quality (Upadysheva et al., 2018). For the plums the question of the stock influence on the fruit quality is not studied enough. The plum hybrid rootstock forms either are self-sterile or have small non-edible fruit with

bitterness. This may cause the deterioration of grafted scion fruit taste and properties. The purpose of our work is to study the productivity and plum (*Prunus rossica* Erem.) fruit quality depending on the stock-scion combination.

Scientific hypothesis

The productivity, the plum (*Prunus rossica* Erem.) fruit quality and their biochemical composition depending on the stock-scion combination are not studied enough. We have checked the influence of the stock on the formation of productivity, quality and nutritional value of *Prunus rossica* Erem fruit Kubanskaya kometa and Naidena grown in Moscow region conditions. We supposed that on the base of the field and laboratory experiments the optimal stock for each breed that will provide the high productivity and valuable biological active substances accumulation in the plum fruit in comparison with the traditional seedling rootstock will be found.

MATERIAL AND METHODOLOGY

The field researches were held in 2017-2018 on the experimental plum *Prunus rossica* Erem. plantations of Federal State Budgetary Scientific Institution "All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery", Moscow region (Figure 1). The plantation overall area is 0.5 ha. The garden of intensive type is set out using the pressed scheme. The soil in the row spacing is agrogenic. The experimental researches object was the fruit of plum Kubanskaya kometa and Naidena breeds grafted on four stocks: 13-113, Novinka, OPA-15-2, *Prunus cerasifera* Ehrh alycha seedling (standard). Under study there were 8 scion-stock combinations totally, not less than 6 trees in each combination were studied. The biochemical researches were held in the Laboratory of Physiology and Biochemistry of Federal State Budgetary Scientific Institution "All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery".

The determination of the productivity and fruit weight and the sensor evaluation

In the period of fruit ripening the trees productivity was determined by the fruitage weighing from each tree in five-time repetition. The average fruit weight and the stone-fruit weight relation were determined by the weighing of 100 fruits in three-time repetition. The sensor evaluation was fulfilled by the group of high qualified specialists for the evaluating products. They estimated three main quality parameters: taste (sweet, sour, with bitterness), after taste and fruit external appearance – the form, colour, the surface condition.

Chemicals

All chemical substances chosen for the analysis were of analytical sort and were bought from Sigma Aldrich (USA).

Sample preparation

From average 1000 g probe 100 g fruit without stone were prepared and extracted by double-distilled water (to determine antioxidant activity) and metaphosphoric acid (for ascorbic acid determination) with the help of highspeed homogenizer (10 000 rpm, 1 min, UltraTurrax T25 Basic, IKA). After centrifugation at 4000 g (Sigma, Germany) within 10 min the supernatant was used for measuring. The extraction as well as the measurements were held in three-time repetition.

Basic chemical analyses

Studied biochemical parameters: dry matter - by drying samples to constant weight at $t = 100$ °C. Titratable acidity. Total titratable acidity (TTA) – titration with 10 N NaOH – was estimated by alkalimeter and expressed in $\text{g}\cdot\text{kg}^{-1}$ malic acid equivalent of fresh matter. The soluble solids content (SSC) was expressed by the index of refraction ($^{\circ}\text{Bx}$). Content of sugars (glucose, sucrose, fructose) by methods of Bertrand (Ermakov, Arasimovich and Jarosh, 1987).



Figure 1 Plum plantations: Left – blossoming garden, row spacing – agrogenic soil. Right – fruitage, Kubanskaya kometa breed.

Ascorbic acid (AsA) determination

Ascorbic acid determination was held using HELC method (Stan et al., 2014), the chromatograph KNAUER (Germany) was used. Chromatographic conditions: HELC column Silasorb C18 (5 mkm), 150 x 4.0 mm (Biohimmac, Russia), the column temperature is 25 °C, flow speed 1.0 mL.min⁻¹, the detector UV, the wave length $\lambda = 251$ nm, the mobile phase MeOH: water – 5:95 (r./r.), aliquote for injections 20 mL, the retention time Rt = 4.4mm.

Antioxidant activity (AA) determination

Antioxidant activity was measured by the Brand-Williams et al. (1995) method using a compound DPPH[·] (2,2-diphenyl-1-pikrylhydrazyl). The spectrophotometer Thermo Helios γ (Thermo Fisher Scientific, made in England) was used. The homogenized by the distilled water samples were put on the shaker Lab-PU-01 (Russia) for 8 hours, and then they were filtered, and the antioxidant activity was measured in 10 minutes after interaction between the extract and reagent at wavelength 515 nm. The calculation of antioxidant activity values was fulfilled using the formula:

$$\text{Inhibiting DPPH} = (\text{AC} - \text{AAt}) = \text{AC} / 100 (\%),$$

Where:

AC – DPPH solution absorption; AAt – absorption at the antioxidant presence.

Three-time repetition.

EDS - analysis

The chemical composition of the basic ash components (Na, P, S, K, Mn, Fe, Mg, Ca, Al, Si, Cl, Zn, Se, Mo) was determined by the method of energy dispersive spectrometry (EDS) on the analytical raster electron microscope JEOL JSM 6090 LA. The microscope solution is 4 nm at accelerating voltage 20 kV (secondary electrons image), zooming is from x 10 till x 10 000. While performing the elemental analysis the working distance (WD) is 10mm. Energy-dispersive spectrometer allows to carry out the quantitative X-ray microanalysis with the desired analysing area: in a point or areally, and to receive the maps of elements allocation. X-ray microanalysis data are presented in the form of standard protocols which contain the microstructure picture of the sample under

study, the table of the data in weighting and atomic correlation, spectra and histograms. The spectrum example is shown in Figure 2.

Taking into consideration the spectrum lines intensity the concentration of the desired element can be determined. The fractional accuracy of the chemical analysis is spread in the following way: at the element concentration from 1 till 5% the accuracy is less than 10%; from 5 till 10% the accuracy is less than 5%; at the element concentration more than 10% the accuracy is less than 2%. 100 ash areas of each sample were studied. The local analysis is 3 mm, the scanned area is not less than 12 μm .

Statistic analysis

All analyses were three-time repetition. Results were expressed as mean values ($n = 3$) \pm standard deviation (SD).

To determine the differences – significance between the data one-way and two-factor experience ANOVA test was used ($p < 0.05$) via the program Statgraphics Centurion XV (USA).

RESULTS AND DISCUSSION

As a result, it was determined that the plum trees productivity at average for the period of 5 years was 14.2 kg/tr. and depending on the stock varied from 9.2 kg/tr. (Naidena on seedlings) to 21.8 kg/tr. (Kubanskaya kometa on OPA-15-2). Higher than the average value this parameter was at the trees of scion Kubanskaya kometa grafted on the stocks 13-113 and Novinka. It should be mentioned that scion Novinka productivity depending on the stock varied in less degree (Table 1). The influence of stock on the productivity of plum trees was noted in (Grzyb, Sitarek, and Kozinski, 1998; Magyar and Hrotkó, 2006).

Scions under study belong to large-fruited group with the fruit weight more than 30 g. The single fruit weight of scion Kubanskaya kometa with average value of 32 g depending on the stock was changing from 29.5 g (seedlings) till 34.8 g (OPA-15-2). The fruits of the scions under study were significantly bigger because of stocks OPA-15-2 and Novinka influence. Scion Kubanskaya kometa stone separated from the flesh badly, its portion in the fruit weight was 7% at average with variation depending on

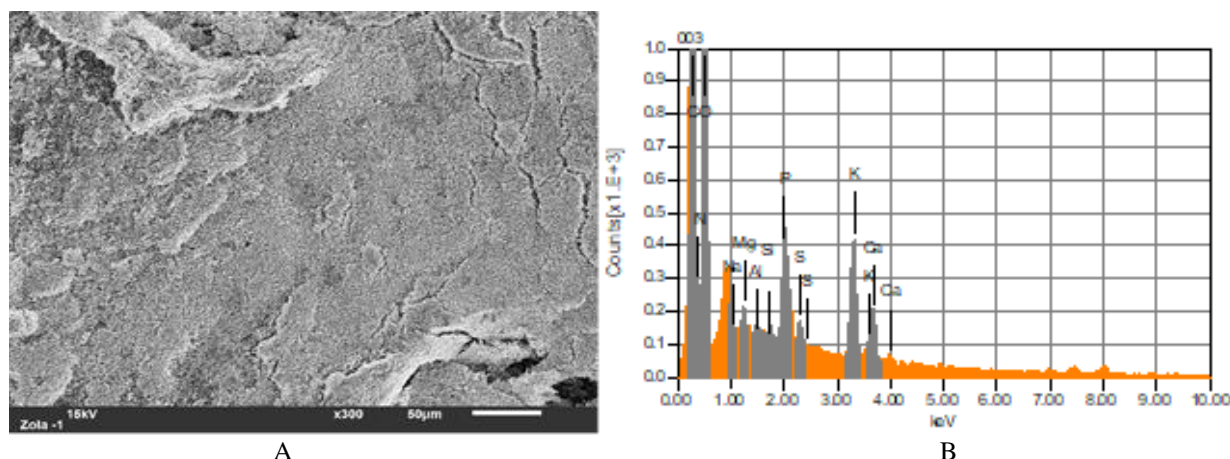


Figure 2 The microstructure picture of the sample under study (A) and the general view of the X-ray spectrum lines (B).

Table 1 The productivity, fetal mass and organoleptic estimation of *Prunus rossica* Erem. fruits depending on the stock at average in 2017 – 2018.

Stock-scion combination	Parameters under study		
	Productivity (kg/tree)	Average fruit Weight (g ±SD)	Degustation evaluation, point
Kubanskaya kometa/ 13-113	16.8± 2.41	31.22 ± 0.41	4.1
Kubanskya kometa/ Novinka	19.4± 1.19	32.33 ± 0.44	4.6
Kubanskaya kometa/ OPA-15-2	21.8 ± 2.54	34.82 ± 0.51	4.8
Kubanskaya kometa/ Seedlings	11.7 ± 1.87	29.54 ± 0.31	4.0
<i>P.cerasifera</i> Ehrh	10.9 ± 2.07	28.64 ± 0.61	4.1
Naidena/ 13-113	11.8 ± 2.17	32.01 ± 0.41	4.3
Naidena/Novinka	12.3 ±1.25	32.53 ± 0.61	4.5
Naidena/OPA-15-2	9.2 ± 0.87	28.07 ± 0.73	4.0

from 6.8% (Novinka) to 7.5% (seedlings).

At scion Naidena the stone was slightly smaller, separated from the flesh better and was nearly 6% for all stock-scion combination. According to the degustation results the fruits of the scions under study were characterized by the harmonized sore-sweet taste and smell (4.0 – 4.8 points), no taste deterioration and bitterness were observed on either of the stocks. The best degustation evaluation parameters were found at the combinations of Kubanskaya kometa on stocks OPA-15-2 and Novinka, Naidena on OPA-15-2 (more than 4.5 points). The plum (*P. rossica* Erem.) fruits were characterized by the high content of soluble solids and sugars which depended on the stock-scion combination. The solutable solids content varied from 11.2% (Kubanskaya kometa on seedlings) to 15.2% (Naidena on OPA-15-2) at average value of 12.9 % (Figure 3.).

However, there is conflicting evidence that confirm that the solids soluble content/titratable acidity ratio weren't affected by soil type or rootstock (Ratoa et al.,2008). The sugars content in the fruits was within 7.2 – 10.0%. The sugars higher accumulation was found at scion-stock combinations Naidena on OPA-15-2 (10%) and on Novinka (9.3%) (Figure 4).

The high positive correlation (r = 0.92) was marked between soluble solids and sugars content. While evaluating the stock influence it should be noted that the soluble solids content on stock OPA-15-2 is on 13 – 20% higher in comparison with alycha standard seedlings. The received data correlate with Hatton et al. (2015) researching results about the stock influence on the plum trees productivity and the apricot fruits productivity and quality (Milosevic et al., 2011) and plum fruits biochemical parameters change (Świerczyński and Stachowiak, 2009).

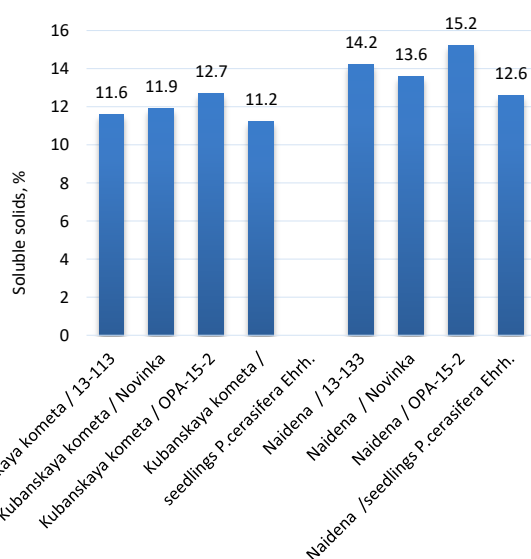


Figure 3 Soluble solids content in plum fruits.

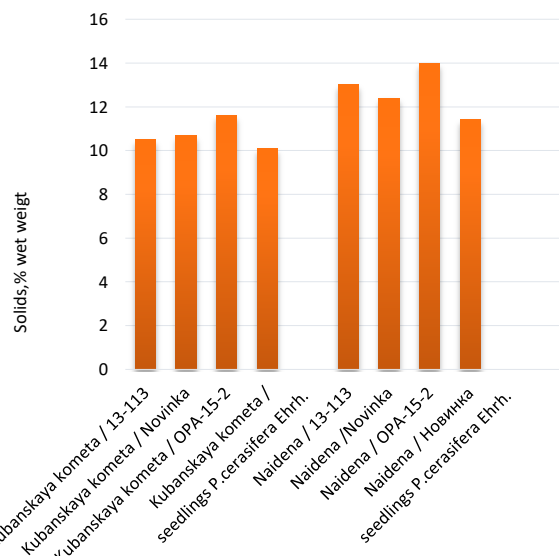


Figure 4 Solids content in plum fruits of *Prunus rossica* Erem., depending on the stock at average in 2017 – 2018.

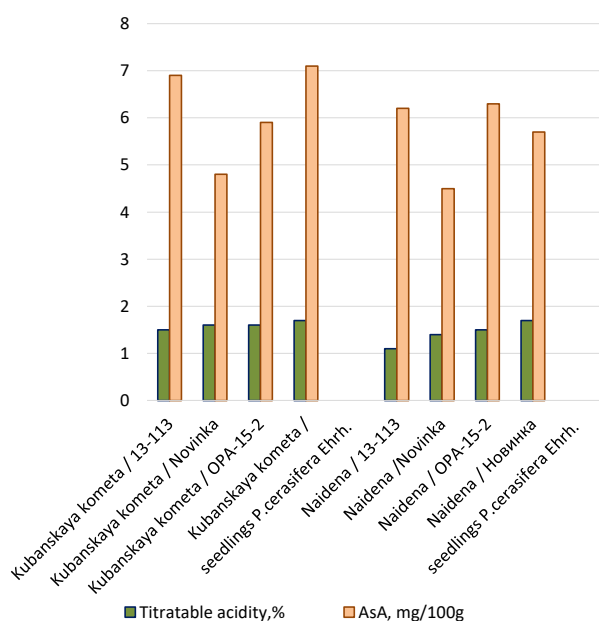


Figure 5 Titratable acidity and ascorbic acid content in plum fruits of *Prunus rossica* Erem. depending on the stock at average in 2017 – 2018.

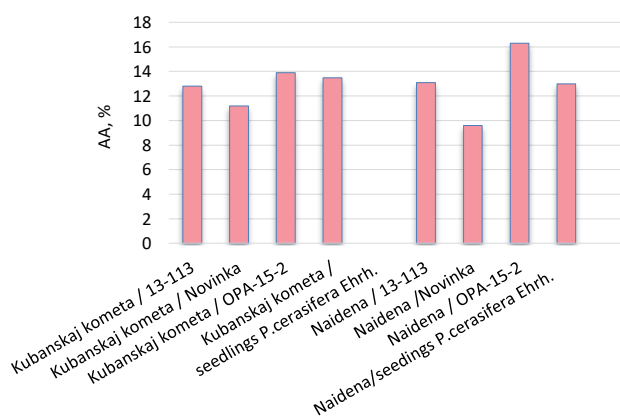


Figure 6 Antioxidant activity in plum fruits of *Prunus rossica* Erem. depending on the stock at average in 2017 – 2018.

Organic acids determined the plum fruits titratable acidity. The scion-stock combination under study showed the titratable acidity variation from 1.1 to 1.7%. The maximum values of the plum fruits titratable acidity (1.6 – 1.7%) were determined for the scion Kubanskaya kometa on the stocks Novinka, OPA-15-2 and on the seedlings *P. cerasifera* Ehrh. Studies performed by Milošević and Milošević (2012) showed that the influence of rootstocks on most of the analysed quality attributes plum was variable because strong and complex interactions, rootstock × cultivar, rootstock × year and cultivar × year, were observed. Several traits, such as yield, soluble

solids/acid content ratio and total sugars/acid content ratio, seem to be harder conditioned in rootstocks. For the scion Naidena on the same stocks the titratable acidity values were maximum (1.4 – 1.7%). On the seedlings the influence on the fruits acidity was expressed by some increase of titratable acidity value and was marked at the level of 1.7% for both scions, the titratable acidity minimum value for the both scions was marked on the stock 13-113 and was marked at the level of 1.5% (Kubanskaya kometa) and 1.1% (Naidena) (Figure 5). The received titratable acid values are lower than in the fruits *Prunus spinosa* L., as it is described in the paper Erturka, Ercislib and Tosun (2009). The researches about the stock influence on the plum fruits titratable acidity change we have not found. AsA content in plum fruits is not relatively high – not more than 7,1 mg/100 g. At average AsA content in scion Kubanskaya kometa fruits is 6.2 mg.100g⁻¹, in scion Naidena fruits – 5.7 mg.100g⁻¹.

It should be mentioned that on the stocks 13-113 and Novinka both scions accumulate the same AsA content. We could not find any papers about the stock influence on AsA content as well, however, the received quantities correspond to the literary data (Gil et al., 2002). The fruits total AA that determines their value for the functional nutrition was nearly 13% depending on the used stock. It varied greatly at scion Naidena: from 9.6% (Novinka) to 16.3% (OPA-15-2), Figure 6. Plums are characterized by a relatively high content of antioxidants. Our results are consistent with those of Najafabad and Jamei (2014). We did not find in the scientific literature information on the effect of stock on the antioxidant activity of plum fruits. But when analyzing the antioxidant activity of cherry plum leaves, we found a significant effect of the stock (Upadysheva and Motyleva, 2019). The correlation between AA and ascorbic acid content in the fruits was determined (r = 0.70). Kalt et al., (1999) reported that the ascorbate content and the antioxidant activity, being negatively correlated (r = -0.80) for strawberries, raspberries and high- and low-bush blueberries. High correlation between antioxidant activity and vitamin C was likely to be found only in fruits that contain high vitamin C such as citrus fruits (Gardner et al., 2000). The high nutritional value of plum fruits is determined not only by the organic bioactive substances content, such mineral components as K, Na, Ca, Mg play an important role (Agbede and Ibitoye, 2007; Motyleva, Simonov and Kulikov, 2017). We analysed the content of 11 elements in the plum fruits ash – K, P, Mg, Ca, Mo, Si, Mn, Fe, Ni, Cu and S (shown in Table 2). K was a prevailing element. The decreasing row of the elements content in the plum fruits ash is the following: K>P>Mg>Ca>Mo>Cu>S>Ni>Fe≈Si>Mn. The proportion of K is from 18.67 (Naidena/OPA-15-2) to 27.48 (Kubanskaya kometa/Novinka); P from 3.35 (Kubanskaya kometa/seedlings *P. cerasifera* Ehrh.) to 6.39 (Kubanskaya kometa/Novinka), Mg from 1.11 (Naidena/OPA-15-2) to 2.43 (Naidena/seedlings *P. cerasifera* Ehrh.) and Ca from 0.39 (Naidena/13-11) to 1.85 (Naidena/OPA-15-2) mass % respectively.

Table 2 The elemental composition of fruit joint of *Prunus rossica* Erem., depending on the stock at average in 2017 – 2018, mass. % in the ash.

Elements	Kubanskaya kometa/stock						Naidena/stock					
	13-113	Novinka	OPA-15-2	seedlings <i>P. cerasifera</i> Ehrh.	Mean \pm SD	Variation coefficient (%)	13-113	Novinka	OPA-15-2	seedlings <i>P. cerasifera</i> Ehrh.	Mean \pm SD	Variation coefficient (%)
K	25.21	27.48	21.19	19.12	23.25 \pm 3.79	16.29	23.45	22.09	18.67	24.98	22.30 \pm 2.7	12.07
P	4.72	6.39	3.36	3.35	4.46 \pm 1.44	32.41	3.38	4.70	3.78	5.15	4.25 \pm 0.8	19.10
Ca	1.16	1.45	1.13	1.24	1.25 \pm 0.14	11.46	0.39	1.20	1.85	1.26	1.18 \pm 0.6	20.88
Mo	1.36	1.11	0.96	0.45	0.97 \pm 0.38	39.40	0.52	1.42	1.96	1.28	1.29 \pm 0.5	45.78
Mg	1.71	1.78	1.26	1.41	1.53 \pm 0.24	15.47	2.34	1.81	1.11	2.43	1.92 \pm 0.6	31.52
S	0.52	0.78	0.52	0.52	0.59 \pm 0.13	21.88	0.23	0.64	0.59	0.41	0.12 \pm 0.2	40.77
Si	0.11	0.18	0.21	0.23	0.18 \pm 0.05	29.86	0.09	0.06	0.12	0.22	0.61 \pm 0.1	36.80
Fe	0.25	0.26	0.18	0.09	0.15 \pm 0.46	32.43	0.11	0.15	0.07	0.26	0.07 \pm 0.1	36.77
Mn	0.08	0.11	-	0.08	0.06 \pm 0.08	39.54	0.01	0.08	0.04	0.13	0.47 \pm 0.1	41.39
Cu	0.54	0.42	0.97	0.68	0.67 \pm 0.31	40.03	0.19	0.59	0.79	0.28	0.23 \pm 0.3	40.55
Ni	0.21	0.39	0.31	0.19	0.24 \pm 0.15	36.17	0.23	0.39	0.25	0.07	0.014 \pm 0.1	35.68
Σ	48.0	51.1	52.7	59.4			59.05	55.9	59.0	58.2		

Note: Results represent mean values (n = 3) \pm SD.

The proportion of the rest elements is not more than 1 mass %. The coefficient of variation of elements in the ash plum Kubanskaya kometa / stock ranges from 15.47% (Mg) to 40.03% (Cu); Naidena/stock ranges from 12.07% (K) to 40.77% (S), which indicates the relative homogeneity of the data. The average values of the coefficients of variation in the ash plum Kubanskaya kometa / stock were found in the elements S (21.88%) and Si (29.86%); in the ash plum Naidena/stock – In the elements Ca (20.88%) and Mg (31.52%). The maximum value of the elements sum was determined in the fruits of scions Kubanskaya kometa and Naidena on the stock seedlings *P.cerasifera* Ehrh.

(59.4 and 58.2) and in the scion-stock combination Naidena/OPA-15-2 (59.0) mass % respectively. At average the maximum value of K, P, Ca, Mo, S, Cu and Fe is found in the plum fruits of scion Kubanskaya kometa in comparison with the value of Mg which is on 37% higher in the scion Naidena. The regularities of the elements content in the ash of both scions on the stocks is identical to the necessary microelements for the human health (Figure 7, 8).

The content of macronutrients in plum fruits is considered in the article (Ertkina et al., 2006).

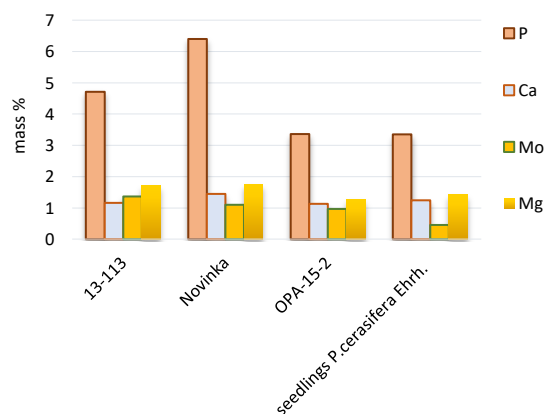


Figure 7 The comparative content of macroelements in the plum fruits ash of scion Kubanskaya kometa on different stocks.

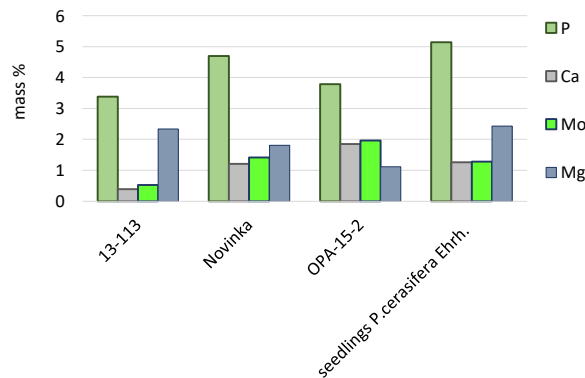


Figure 8 The comparative content of macroelements in the plum fruits ash of scion Naidena on different stocks

CONCLUSION

In the present paper the main focus was given to the study of the essential biochemical parameters that characterize the nutritional and dietic value of plum fruits (*Prunus rossica* Erem.) of different scion-stock combinations. As the result of the researches the stock influence on the trees productivity, the plum fruits (*Prunus rossica* Erem.) weight and chemical composition was identified and the limits of soluble solids, sugars and ascorbic acid content variation depending on the used scion/stock combinations were determined. Under the stock OPA-15-2 influence higher content of soluble solids, sugars and ascorbic acid accumulated in the fruits. While grafting on alycha seedlings the reduction of the parameters under study was observed. Using the data of the field and laboratory researches the conclusion can be made that the optimal stock for scions Kubanskaya kometa and Naidena is OPA-15-2 may be a better solution to achieve higher yields and better fruit. The results received while working at this paper give new information about the stock influence on the biochemical characteristics of the scions.

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