

# Blue honeysuckle introduction and breeding in the Central Siberian Botanical Garden (Novosibirsk, Russia)

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**Abstract.** The use of the blue honeysuckle *Lonicera caerulea* subsp. *altaica* (*Caprifoliaceae*) for breeding resulted in the novel forms exceeding the parent ones in early maturity, fruit mass, biologically active compounds content and the crown shape allowing the mechanized harvesting.

## 1 Introduction

Subsection *Caerulaea* Rehd., or blue honeysuckles (*Lonicera* genus of the *Caprifoliaceae* Juss. family) according to the M.N. Plehanova's classification [1], in Eurasia consists of three endemic diploid species *Lonicera iliensis* Pojark., *L. edulis* Turcz. ex Freyn, *L. boczkarnokowae* Plekhanova nom. nov. (= *L. regeliana* Boczkarn.) and a tetraploid species *L. caerulea* L., embracing seven subspecies of *L. caerulea* subsp. *caerulea*, *L. caerulea* subsp. *emphylocalyx* (Maxim) Plekhanova comb. nov., *L. caerulea* subsp. *altaica* (Pall.) Plekhanova comb. nov., *L. caerulea* subsp. *pallasii* (Ledeb.) Browich, *L. caerulea* subsp. *stenantha* (Pojark) Hult. ex Skvortsov, *L. caerulea* subsp. *kamtschatica* (Pojark.) Plekhanova comb. nov., *L. caerulea* subsp. *venulosa* (Maxim) Worosh.

Blue honeysuckle is spread in the taiga zone of Eurasia and North America. On most of their area their berries have bitter taste and are not edible. Till the middle of the 20<sup>th</sup> century the species of the subsection were not considered even as a potentially edible plant. Only in the Far East [2], in the north of China and the island of Hokkaido in Japan the fruits of *L. caerulea* subsp. *kamtschatica*, *L. caerulea* subsp. *venulosa*, *L. caerulea* subsp. *emphylocalyx* and *L. boczkarnokowae* were used as food [3, 4]. The natural populations of *L. caerulea* subsp. *kamtschatica* and *L. caerulea* subsp. *Venulosa* became the source for breeding the first cultivars of blue honeysuckle. However, most of the cultivars of the Far East provenance decreased their productivity in the steppe and forest-steppe areas of the West Siberia due to the continental climate. In the region the species *L. caerulea* subsp. *altaica* seemed to have better prospects in the intensive breeding programs, as the species combines a number of economically valuable traits, such as high productivity, early maturity, drought resistance, firm attachment of compound fruits and high bioflavonoid content. The dominance of bitter-tasting fruits in the offspring made it difficult to use the *L.*

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*caerulea* subsp. *altaica* as initial source material, thus urging to find natural populations of the subspecies with prevalence of individuals without the bitter taste of their fruits.

In ethnic medicine the blue honeysuckle species have been known for a long time as medicinal plants [5]. Numerous studies confirmed the beneficial for human health effects of *L. caerulea* berries, such as antioxidant activity, antibacterial, immunological [7], anti-inflammatory, neuroprotective, antitumor and antidiabetic activities of the blue honeysuckle preparations [8]. The main components of biologically active phenolic compounds (PC) in *L. caerulea* fruits are such flavonoids as anthocyanins, flavonols and flavans, hydroxycinnamic acids (HCA) and flavans [8].

Differential qualitative and quantitative fruit PC contents were found in different *L. caerulea* varieties and genotypes [9]. The fruit PC profiles were also found to be affected by the plant growing environment [9]. It is known that successive selection of the blue honeysuckle plants by their fruits sensory properties, size, yield and cold resistance may result in decreasing both the total PC content as well as the ratio of their specific groups [10]. Therefore the information about the fruit PC content is indispensable for breeding and introduction of varieties obtained using the species of the other geographical provenances.

The last decades saw the active use of blue honeysuckle in agricultural production in temperate regions [3, 5, 11]. Since 2016 in Russia in the West Siberia agricultural plantations of blue honeysuckle are started every year in the Tomsk and Novosibirsk regions, currently totaling 300 ha. So the situation urges for finding new source material for selection, focusing on creating and breeding of varieties suitable for mechanized harvesting.

The aim of the work was to compare agriculturally valuable traits, including the fruit polyphenolics profiles of the blue honeysuckle varieties bred by the Central Siberian Botanical Garden (CSBG) in the forest-steppe near the Ob River (Novosibirsk, Russia).

## 2 Material and methods

Fruit mass, taste and attachment were determined in fruits of the 17 blue honeysuckle forms bred by the CSBG on the right bank Ob River area in the forest-steppe by using standard methods [12].

The individual PC compounds in fruits was determined for three varieties of different ecological and geographical provenances, bred by the Lisavenko Research Institute of Horticulture, namely Zolushka (*L. caerulea* subsp. *Kamtschatica*), Pamyati Guidzyuka (*L. caerulea* subsp. *venulosa*), Berel (a hybrid between *L. caerulea* subsp. *altaica* and *L. caerulea* subsp. *kamtschatica*), as well as ten CSBG selected *L. caerulea* forms. Technically ripe fruits were collected and frozen at  $-18^{\circ}\text{C}$ .

The flavonoid and HCA content was estimated using HPLC technique. Identification of individual compounds of the analyzed in fruit extracts, and estimation of their contribution into the total PC content was performed by using HPLC-MS system. The system included the liquid chromatographer «Agilent 1200» (with diode-matrix detector) and the hybrid quadrupole time-of-flight mass-spectrometer micrOTOF-Q (Bruker Daltonics, USA); the Zorbax SB-Aq column of  $2.1 \times 150$  mm and  $3.5 \mu\text{m}$  in diameter; 2% HCOOH-ACN with a linear gradient of acetonitrile (ACN) from 5 to 25% from 0 till 15 min, and from 25 to 90% from 20 till 25 min. The flow rate was 0.2 ml/min. The UV-Vis detection was carried out using the five wavelengths of 255/16, 340/32, 370/80, 460/80 and 650/80 nm. The working parameters of the MS detection were as following: ionization with electrospray under atmospheric pressure (API-ES), scanning for anions in the  $m/z=100-1000$  range, the desiccating gas (nitrogen) flow rate of 8 L/min with its temperature of  $240^{\circ}\text{C}$ , and the pressure of 2.0 Bar at the ion funnel.

Comparative analysis of the individual PC composition of the fruit extracts was performed on the basis of the integral intensity of the chromatographic signal of a

component: for anthocyanins at wavelength of 460±40 nm, and for HCA, flavonols and flavons at 340±16 nm.

By using as reference standards the anthocyanin content in the blackberry extracts, and rutin and chlorogenic acid reference samples we determined the anthocyanin content as equivalent to cyanidin-3-glucoside, and the flavonols and flavons contents as equivalent to rutin, whereas the HCA content was calculated as equivalent to the chlorogenic acid, using the formula  $C_x = 100 \cdot S_1 \cdot C_{ref} \cdot V / S_{ref} \cdot m$ , where:  $S_1$  is a peak area of an individual component in the analyzed sample;  $C_{ref}$  is a concentration in a reference standard;  $V$  is the volume of the extragent, ml;  $S_{ref}$  is a peak area in a reference sample; and  $m$  is an aliquot mass, mg.

The flavonoid content was determined as a sum of quercetin glucosides, whereas the flavon content was estimated as the sum of luteolin and apigenin contents together with their free aglicons. The HCA derivatives were estimated as sum total of neochlorogenic, dicaffeoylquinic, chlorogenic acids and their isomers.

The relative standard deviation for repeatability while determining phenolic compounds was s.d.=0.010.

### 3 Results and discussion

The CSBG has been developing the introduction population of blue honeysuckle since 1970. The first sample plants were collected from the natural populations in the Altai Mountains. During the following years the collection increased due to the breeding material provided by the Lisavenko Research Institute of Horticulture, its experimental station in the Bakchar village (Tomsk region, Russia) and the N.I. Vavilov Research Institute of Plant Genetic Resources (Saint-Petersberg, Russia). In 1998 a field breeding plot was set up, where seedlings resultant from the spontaneous pollination of samples from the natural populations in the Altai Mountains were planted, namely no.39, no.45 and no.72. Then from these seedlings some promising varieties with big (up to 2.0 g) firmly attached fruits with dissert taste and strong peel (Table 1) were selected. The breeding program was also targeted at selecting the varieties with the shrub crown well above the ground surface, moderate branch density, flexible skeletal branches and with plant height and diameter not exceeding 1.5 m, suitable for mechanized harvesting.

**Table 1.** Fruit properties of selected forms obtained by spontaneous pollination of *L. caerulea* subsp. *Altaica* samples

Variety	Fruit mass, g		Taste	Fruit attachment, score
	Mean	Maximum		
Tsarevna (3-3-03)	1.3	1.5	Sour-sweet	3
Marmaladnaya (3-10-03)	1.4	1.5	Sweet	3
Elan (4-5-03)	1.1	1.6	Sweet-sour	4
Capella (4-7-03)	1	1.4	Sour-sweet	3
Krepkaya (4-8-03)	1.1	2.0	Sweet-sour	4
Verhkubinskaya (4-10-03)	1.2	1.5	Sour-sweet	4
Multa (4-16-03)	1.1	1.9	Sweet-sour	4
Yadrenaya (9-4-03)	1.3	1.9	Sour	4

Simultaneously the hybridization of the varieties of distant geographical provenances was carried out: *L. caerulea* subsp. *altaica* (Salute cultivar), *L. caerulea* subsp. *venulosa* (Tomichka, Pamyati Guidzyuka, Parabelskaya cultivars) *L. caerulea* subsp. *kamtschatica* (Goluboye vereteno, Zolushka, Kaamchadalka cultivars), the hybrid between *L. caerulea* subsp. *altaica* × *L. caerulea* subsp. *kamtschatica* (Berel cultivar). As a results of the cross-pollination of three subspecies of blue honeysuckle the choice forms were obtained (Table 2), exceeding the parental forms in fruit mass and productivity, as well as in biologically active compounds’ content. The crossing combinations, resulting in the positive transgression in biologically active phenols content, were also found [13].

**Table 2.** Fruit properties of the selected blue honeysuckle varieties resultant from the crossing of the varieties of distant geographical provenances.

Variety	Fruit mass, g		Taste	Fruit attachment, score
	Mean	Maximum		
Doch Zolushki (1-7)	1.1	1.4	Sourish-sweet	3
Freya (2-48)	1.2	1.6	Sourish-sweet	2
Golubaya mechta (3-22)	1.1	1.4	Sourish-sweet	3
Aina (3-25)	1.0	1.3	Sourish-sweet	4
Vega (3-28)	1.2	1.4	Sourish-sweet	2
Ayula (3-31)	1.0	1.3	Sourish-sweet	3
Uimonskaya (3-32)	1.0	1.2	Sourish-sweet	3
2-11	0.9	1.8	Sweetish-sour	3
2-23	0.7	1.0	Sweetish-sour	3

The individual composition of PC in the fruits of the selected *L. caerulea* forms was characterized by HPLC (Table 3). The anthocyanins are known to be the main bioflavonoids in honeysuckle fruits [8, 10, 13]. We found that the main anthocyanin in the studied *L. caerulea* fruit samples was represented by cyanide-3-glucoside (up to 91% of total bioflavonoids), whereas cyanidin-3,5-diglucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, peonidine-3-glucoside were the minor components. Chlorogenic, neochlorogenic and dicaffeoylquinic were identified among the hydroxycinnamic acids; in flavonols and flavonols group glucosides and rutinosides of quercetin, luteolin and apigenin were identified.

The maximal anthocyanin content (875–1079 mg/100 g) was found in the fruits of such varieties as Multa, Yadrenaya, Marmaladnaya, Tsarevna, No. 2-11 and No. 2-23, obtained with participation of *L. caerulea* subsp. *altaica*, as well as Pamyati Guidzyuka, obtained with participation of *L. caerulea* subsp. *venulosa*. The minimal content (510–574 mg/100 g) was found in the varieties of the Kamchatka provenance, including the fruits of the hybrid between *L. caerulea* subsp. *altaica* × *L. caerulea* subsp. *kamtschatica* (Berel cultivar).

Quercetin rutinoside (rutin), chlorogenic and dicaffeoylquinic acids were present in extracts from the fruits of all studied samples. The selected form Tsarevna had no flavonols in its extracts. The composition profiles of minor components varied. Earlier significant differences in the major PC content in fruits produced by some *L. caerulea* subspecies were found [13]. The PC composition variation in fruits of the selected *L. caerulea* varieties most

likely depends on the contribution of the initial forms of different provenances which grow on the CSBG experimental plot.

**Table 3.** Flavonoid and hydroxycinnamic acids content in fruits of different *L. caerulea* varieties, mg/100 g (fresh mass)

Phenolic compounds	Varieties and selected forms*										
	1	2	3	4	5	6	7	8	9	10	11
Cyanidin glucoside	845	490	507	548	875	690	692	1038	800	862	924
Chlorogenic acid	95	47	50	56	82	58	55	73	78	72	107
Neochlorogenic acid	6	3	4	3	5	4	5	0	0	0	6
Quercetin glycoside (m.m.=742.19)	2	0	0	0	0	0	3	0	0	0	0
Quercetine glycoside (m.m.=756.20)	3	0	0	2	4	0	3	5	3	3	0
Luteolin glycoside (m.m. = 596.14)	0	0	0	0	0	0	0	3	0	0	0
Quercetin glycoside (m.m.=610.15)	18	0	0	7	0	8	17	0	0	0	13
Quercetin glycoside (m.m.=610.15)	37	18	15	23	7	28	39	4	5	10	31
Luteolin glycoside (m.m. = 580.14)	0	0	0	1	0	0	0	10	0	0	4
Quercetin glycoside (m.m.=464.10)	0	4	3	6	20	5	0	21	6	5	5
Luteolin rutinoside (m.m. = 624.17)	0	0	0	4	10	0	0	16	0	0	0
Luteolin glycoside (m.m. = 448.10)	10	6	7	9	13	23	12	13	6	0	8
Dicaffeoylquinic m.m.=516.11	17	12	14	37	28	43	22	37	25	11	24
Apigenin glycoside (m.m. = 432.10)	0	0	0	0	0	0	0	5	0	0	0
<b>Anthocyanins</b>	<b>884</b>	<b>510</b>	<b>526</b>	<b>574</b>	<b>918</b>	<b>704</b>	<b>716</b>	<b>1079</b>	<b>832</b>	<b>898</b>	<b>947</b>
<b>HCA**</b>	<b>117</b>	<b>62</b>	<b>69</b>	<b>98</b>	<b>115</b>	<b>105</b>	<b>82</b>	<b>110</b>	<b>103</b>	<b>83</b>	<b>137</b>
<b>Flavonols</b>	<b>60</b>	<b>22</b>	<b>18</b>	<b>38</b>	<b>30</b>	<b>40</b>	<b>62</b>	<b>30</b>	<b>14</b>	<b>18</b>	<b>49</b>
<b>Flavons</b>	<b>10</b>	<b>6</b>	<b>7</b>	<b>14</b>	<b>23</b>	<b>23</b>	<b>12</b>	<b>46</b>	<b>6</b>	<b>0</b>	<b>12</b>
<b>Total polyphenols</b>	<b>1072</b>	<b>655</b>	<b>620</b>	<b>724</b>	<b>1087</b>	<b>872</b>	<b>872</b>	<b>1266</b>	<b>954</b>	<b>999</b>	<b>1146</b>

Note: \* 1 – Pamyati Guidzyuka, 2 – Zolushka, 3 – Doch Zolushki, 4 – Berel, 5 – Multa, 6 – Capella, 7 – Krepkaya, 8 – Yadrenaya, 9 – Marmeladnaya, 10 – Tsarevna, 11 – 2-23; \*\* HCA – hydroxycinnamic acids.

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