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Reviewed Paper

# Biological traits of clone varieties of *Malus sieversii* from the *ex-situ* collection in Almaty

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## Abstract

*The mountain forest massifs of Ile Alatau and Jongar Alatau in the Republic of Kazakhstan are essential areas for agrobiodiversity concentration. These areas are home to over 100 species of wild fruit, belonging to 30 genera and 13 families. Many of these species are relict and endemic, and the wild apple tree, Malus sieversii (Ledeb.) M. Roem., is of global significance. In this study, 23 clones of M. sieversii selected from the Ile Alatau and Jongar Alatau mountains were evaluated using various phenological, morphological, and biochemical traits. The different clone varieties showed significant variation in their phenological, morphological, and biochemical traits. The harvest dates of the clones ranged from August 5 to October 10, while the fruit weight varied from 7.47 to 170 g. The fruit shape of the genotypes was round, conical, and oblong. Many clones of M. sieversii contained high levels of bioactive substances. This study can contribute to the commercialization of promising M.sieversii clones that are rich in bioactive compounds for commercial cultivation and breeding programs.*

**Keywords:** Wild apple, Alatau, phenological properties, biochemical traits, cider

## Introduction

Between 1973 and 2005, wild fruit forests in Kazakhstan were surveyed to study and preserve them under the leadership of the Academician of the National Academy of Sciences of Kazakhstan A.D. Dzhangaliev. The result was the creation of a collection fund of wild fruit plants at the Main Botanical Garden in Almaty. The main goal of this collection was to create a unique gene pool of economically valuable wild relatives of fruit crops, especially *Malus sieversii* (Ledeb.) M. Roem and *Prunus armeniaca* L. These plants are vital for the conservation of degraded wild fruit forests in Kazakhstan and for effective use in breeding and agricultural activities in the country (Dzhangaliev , 2007).

The collection gene pool of wild fruit plants was created through the purposeful work of several generations of scientists who mobilized the genetic resources of wild fruit plants in Kazakhstan. The collection contains a globally unique indigenous species, the *M. sieversii*, which is recognized as endangered in Kazakhstan according to the Red Book of Kazakhstan (Isaev, 2014). The area of apple forests in the central part of Ile Alatau decreased by 80% from 1932 to 2007, and in Jongar Alatau since 1960 by 28% (Dzhangaliev, 2007, Hokanson et al.,1999). Therefore, it was necessary to organize works on the selection of forms of *M. sieversii* from natural places of growth to preserve their genetic diversity and as a world gene center of origin of cultivated apple trees and development of industrial horticulture.

The clone varieties of *M. sieversii* selected by A.D. Dzhangaliev are promising for restoring native fruit forests of the Republic of Kazakhstan and expanding the area of industrial apple culture. They represent valuable breeding material of global importance, characterized by a longer productive period compared to cultivated varieties, less labor-intensive tree care, frost resistance, stress tolerance, and high yield (Dzhangaliev, 1977, 2003).

It's important to note that the uniqueness of *M. sieversii* of Kazakhstan as the progenitor of most of the world's varietal diversity was confirmed by foreign scientists (Hokanson et al.,

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1999, Morgan et al., 2002, Harris et al.,2002, Forsline at al., 2003). We have used 8 different markers called ISSR to identify the genetic diversity of 13 different clone varieties of *M. sieversii* from the Jongar population of the Main Botanical Garden's introduction collection and 31 samples from three different populations. All the data was analyzed using the iMEC program as described in the study conducted by Shadmanova et al. in 2019.
The aim of this study was to evaluate 20 clone varieties of *M. sieversii* from the *ex-situ* collection for morphological, phenological, and biochemical traits for the selection and restoration of degraded forests.

## Material and Methods

The study was conducted at the Institute of Botany and Phytointroduction in Almaty over a period of three years (2019–2021) in the *ex-situ* collection of wild fruit plants from Kazakhstan. The collection contains 170 clone varieties of *M. sieversii* from different mountain ranges: Tarbagatay, Jongar Alatau, Ile Alatau, Ketpentau, and Karatau. For the study, twenty *M. sieversii* clone varieties were selected from Ile and Jongar mountain ranges (Table 1). Clones from different populations were grafted onto wild apple (*M. sieversii*) seedlings and planted in an orchard using a 6x4 m scheme in 1992. Twenty fruits were harvested randomly from each genotype in three replications at the stage of physiological ripeness during the 2019 and 2021 seasons in the experimental collection.
Phenological properties such as the start and the end of flowering were recorded according to the recommendations of the International Working Group for Pollination (Wertheim,1996). Phenotypic characterization descriptors such as flesh color, flesh firmness, flesh flavor, fruit ground color, fruit overcolor, fruit shape, fruit ribbing, and fruit stalk length were measured. The phenotypic characteristics were determined for a period of three harvest seasons for 20 fruits from each tree in three replicates. The data were collected based on the UPOV guidelines (International Union for the Protection of New Cultivars of Plants, 2005) and Djangaliev, 2008.
The three–year physicochemical characteristics such as fruit weight, dry matter, total sugar, titratable acids, sugar–acid index, and leucoanthocyanidins were also described. The fruit mass was determined by weighing using an analytical balance. The biochemical compositions of 20 apple clone varieties were studied. The fruits for analyses were harvested at the stage of picking maturity. Fresh apple samples were used in biochemical analyses. Total sugar was determined by Bertran's method with minor modifications (Ermakov, 1987), titratable acidity was determined by titrating apple samples with 0.1 M NaOH in the presence of phenolphthalein and leucoanthocyanidin was determined by butanol- HCl assay (Hagerman et al., 2000). The data presented in this study represent the means of two years of experiments, each consisting of three replicates of 10 fruits. Standard deviations were calculated to evaluate the variability in the data.

## Results

*Phenological properties*

The flowering season of different apple clone varieties was assessed based on preliminary results over a three-year period. The onset of flowering was recorded to be earliest in 'Asya' on April 14 and latest in 'Ketpen' on April 30. Figure 1 shows the distribution of the apple varieties based on their flowering patterns. The apple clone varieties were categorized into three groups based on their full flowering time: extremely early, middle, and late. The extremely early genotypes included 'Asya' and 'Jongar large fruit', while the late ones were 'Ile medium fruit', 'Jongar rootstock', 'Sirga alma', 'Jolak alma', 'Jongar

cider', 'Jongar molonimdi' and 'Ketpen'. Most of the apple clone varieties belonged to the middle flowering group, which included 'Jongar autumn fruit', 'Jongar green fruit', 'Ile honey', 'Askar', 'Jongar kisgilt', 'Jongar fir tree', 'Jongar', 'Jongar crimson', 'Mushabay', 'Usak' and 'Ile'. The highest flowering duration was observed in the clone varieties with a range of 15-19 days ('Jongar kisgilt', 'Jongar', 'Mushabay', 'Jongar crimson' and Jongar large fruit).

Table 1: *M. sieversii* clone varieties, their descriptions of collection sites and years of collection.

| Clone varieties | Mountain/Range | Elevation (m) | Years of collection |
|---|---|---|---|
| Askar | Jongar Alatau/Soldat | 1000-1350 | 1972 |
| Asya | Jongar Alatau/Soldat | 1000-1350 | 1962 |
| Jongar Autumn Fruit | Jongar Alatau/Soldat | 1000-1350 | 1948 |
| Jongar rootstock | Jongar Alatau/Soldat | 1000-1350 | 1990 |
| Jongar kisgilt | Jongar Alatau/Soldat | 1000-1350 | 1990 |
| Sirga alma | Jongar Alatau/Soldat | 1000-1350 | 1946 |
| Jongar fir tree | Jongar Alatau/Samirsin | 1000-1350 | 1990 |
| Jongar | Jongar Alatau/Samirsin | 1000-1350 | 1990 |
| Usak | Jongar Alatau/Samirsin | 1000-1350 | 1990 |
| Jongar cider | Jongar Alatau/Mushabay | 1510-1820 | 1990 |
| Mushabay | Jongar Alatau/Mushabay | 1510-1820 | 1990 |
| Jolak alma | Jongar Alatau/Mushabay | 1510-1820 | 1990 |
| Jongar large fruit | Jongar Alatau/Kara osen | 1250-1600 | 1948 |
| Jongar molonimdi | Jongar Alatau/Antabay | 1250-1600 | 1948 |
| Jongar crimson | Ile Alatau/Kuznezov | 1350-1750 | 1946 |
| Ketpen | Ile Alatau/Kuznezov | 1350-1750 | 1996 |
| Ile green fruit | Ile Alatau/Kara koniz | 1300-1650 | 1990 |
| Ile honey | Ile Alatau/Kara koniz | 1300-1650 | 1990 |
| Ile | Ile Alatau/Kara koniz | 1300-1650 | 1990 |
| Ile medium fruit | Ile Alatau/Kara koniz | 1300-1650 | 1990 |

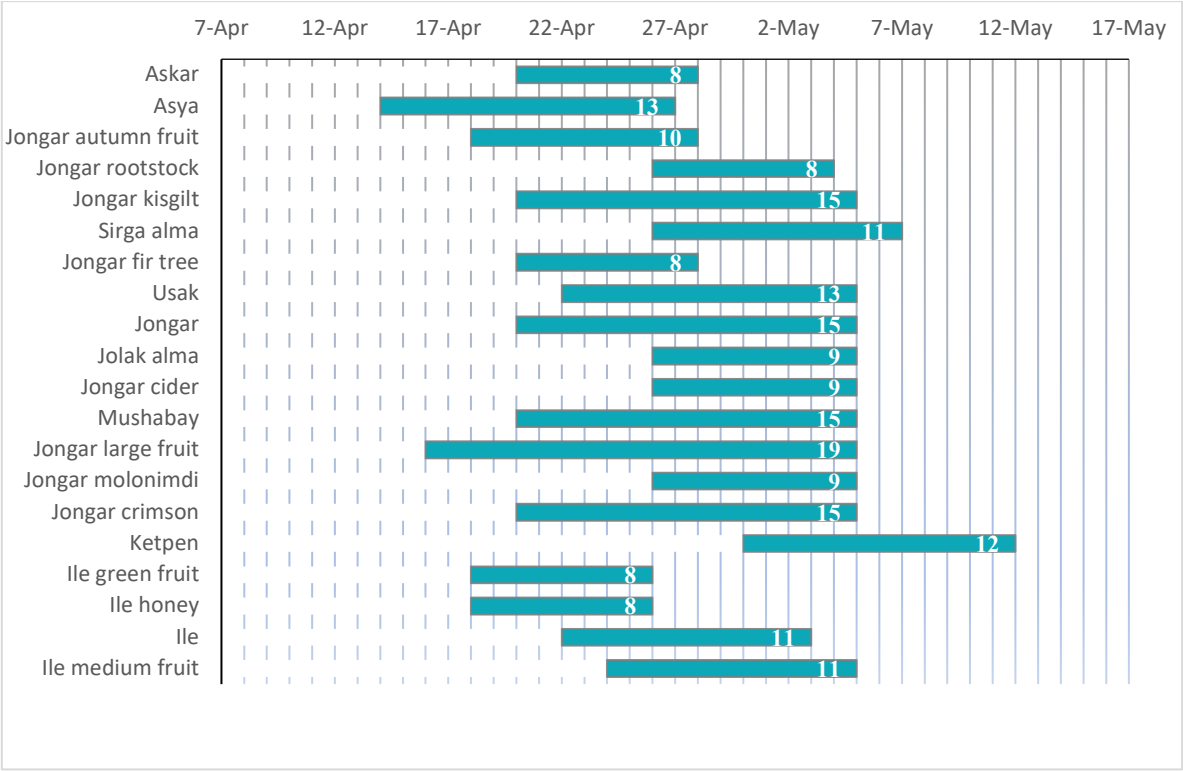

Figure 1: Flowering phenophase of the assessed apple clone varieties

*Biochemical and pomological traits*

*Fruit weight*

The 20 different clone varieties of *M.sieversii* showed significant variations in fruit weight, ranging from 7 grams (Usak) to 170 grams (Askar). The distribution of the fruits based on weight was: very small (7-25 g) – Usak, Jongar rootstock (2), small (26-50 g) – Sirga alma (1), small to medium (51-75 g) – Jolak alma, Mushabay, Red fir tree, Jongar molonimdi, Ketpen, Jongar cider (6), (101-124 g) – Jongar kisgilt, Ile Green fruit, Jongar Large Fruit (3), (126-175 g) – Ile Medium Fruit, Ile, Ile Honey, Jongar, Asya, Jongar Autumn Fruit, Jongar crimson, Askar (8). The variability of fruit weight observed within the experimental three years and expressed as a coefficient of variation varied among the different clones. Sirga alma', 'Ketpen', 'Jongar cider', 'Mushabay', 'Ile' and 'Usak', were unstable for this trait (CV 25, 19.4, 18.6, 18.5, 16.5, 16.4 % respectively) while 'Jongar', 'Jongar Crimson', 'Ile Honey', 'Jongar kisgilt', 'Red Fir Tree', 'Ile Green Fruit', 'Askar', 'Jongar molonimdi', 'Jongar Autumn Fruit' and 'Asya' showed a constant CV ranging from 1.12 to 10 % and did not depend on growing conditions (Table 2).

*Dry matter*

The dry matter (DM) content in the fruits of the evaluated clones varied significantly from 11.4 % (‘Jolak alma’) to 28.8 % (‘Asya’, Ile medium fruit), with the average value for the group of twenty clones being 20.72 %. The clones 'Ile honey', 'Jolak alma', 'Jongar cider', 'Jongar crimson', 'Jongar kisgilt', 'Jongar molonimdi', 'Ile', 'Ile green fruit', 'Usak' had dry matter content at or above the mean value of 20.72, with values ranging from 20.5% to 25.8% (Table 2). The coefficients of variation, which were lower than 9%, indicate that the DM content in the fruits of all clones is stable. High DM content in the fruits suggests that they are suitable for making apple powders.

*Sugars*

The apple cultivars that were studied had a range of total sugar content from 7.75 % ('Jolak alma') to 14.3 % ('Jongar autumn fruit'), with an average value of 11.5%. The highest sugar content in the fruits was found in 'Jongar autumn fruit', 'Ile medium fruit', 'Ile',

'Ile green fruit', 'Ile honey', 'Jongar rootstock', 'Askar', 'Jongar fir tree', 'Jongar crimson', 'Asya', 'Ketpen', 'Jongar cider' (14.3–11.8%). However, clones 'Jongar kisgilt', 'Ile', 'Jongar molonimdi', 'Mushabay', 'Ile honey', 'Jongar cider', 'Ketpen', and 'Sirga alma' did not have a consistent reduction in sugar content over the years of the study, with a coefficient of variation (CV) higher than 17 % (Table 2).

*Titrated acids (TA)*

Titrated acidity is an important measurement for evaluating the quality of apple fruit (Lo Piccolo et al., 2019). The fruits of 'Jongar kisgilt', 'Jongar', 'Askar', 'Jongar crimson', 'Sirga alma', and 'Usak' accumulated the highest amount of these substances in the southeastern region of Kazakhstan, with a range of 0.91 % to 1.57 %. On the other hand, 'Jongar molonimdi', 'Ile green fruit', 'Jongar cider', 'Jongar fir tree', and 'Jongar rootstock' accumulated the lowest amount, with a range of 0.19 % to 0.39 %. The mean value for these cultivars was 0.69 %. The fruits of clones Asya, Jongar, Jongar large fruit, Ile green fruit, Jongar cider, Usak, Sirga alma, Jolak alma, and Ile honey showed stability in this index (ranging from 0.87% to 13.6%). Clone varieties fruits were the most variable, with a coefficient of variation (CV) of over 28 %. Specifically, fruits of 'Jongar rootstock', 'Jongar molonimdi', 'Jongar autumn fruit', 'Jongar kisgilt', 'Ile', and 'Ile medium fruit' showed a high level of variability (Table 2).

*Sugar acid index (SAI)*

The quality, taste, and further utilization of apples depend on the ratio of sugars and acids in them. Sugars determine the sweetness level while titrated acids determine the sourness level. Fruits with a Sugar Acid Ratio (SAI) of less than 20 are sharper in taste and are generally used for processing, such as making cider. Fruits with an SAI above 20 are usually eaten fresh and are considered dessert. Clone varieties bred in Kazakhstan have an average SAI of 21.9, with the 'Jongar molonimdi' apple clone having the highest SAI value of 55.7 and the 'Sirga alma' clone having the lowest SAI value of 7.09. The fruits of 'Asya', 'Ile honey', 'Ketpen', Ile and Ile medium fruit clones had an SAI higher than the group's mean value, indicating a harmonious and balanced taste (21, 22, 23, 27 respectively). These clones are suitable for both fresh consumption and processing. The fruits of cultivars with an SAI value lower than 20, specifically 'Sirga alma', 'Usak', 'Jongar large fruit', 'Jolak alma', 'Jongar', 'Jongar kisgilt', 'Jongar crimson', 'Askar', 'Mushabay', and 'Jongar Autumn fruit' are suitable for making cider if harvested at maturity.

Table 2: Biochemical characterization of clone varieties of *M. sieversii*

| Clone varieties | Weight, (g) | DM | TS, % | TA, % | SAI | Leuco, mg/100 g FW |
|---|---|---|---|---|---|---|
| Askar | 170 (8,1) | 17.8 (8.7) | 12.8 (1,2) | 0,98 (17,9) | 13,1 | 75,7 (15.0) |
| Asya | 135 (10) | 28.8 (5.5) | 12.3 (12,2) | 0.58 (0,87) | 21,2 | 79.2 (12.3) |
| Jongar Autumn fruit | 153 (10) | 14.0 (1.1) | 14.3 (8.6) | 0.75 (31.1) | 19.1 | 58.9 (22.2) |
| Jongar rootstock | 24,27 (13.5) | 18.5 (7.8) | 12,87 (14) | 0,39 (28,8) | 33.0 | 80.9 (17.0) |
| Jongar kisgilt | 109,6 (5.5) | 21.9 (8.3) | 10.1 (17.1) | 0.91 (31.6) | 11.1 | 39.3 (31.1) |
| Sirga alma | 48,6 (25,2) | 20.6 (9.1) | 8.3 (23.9) | 1.17 (11.9) | 7.09 | 184.6 (11.2) |
| Jongar fir tree | 57.75 (7.2) | 18 (6.9) | 12.7 (5.9) | 0.35 (21.4) | 36.2 | 181.5 (27.8) |
| Usak | 7.47 (16.4) | 25.8 (7.5) | 11.5 (9.1) | 1.57 (8.3) | 7.32 | 463 (23.7) |
| Jongar | 134.5 (1.12) | 17.9 (2.4) | 10.56 (3.9) | 0.96 (2.9) | 11.0 | 188 (30.5) |
| Jolak alma | 54.2 (13.9) | 11.4 (7.0) | 7.75 (22.6) | 0.72 (12.5) | 10.7 | 244.3 (4.5) |
| Jongar cider | 74.6 (18.6) | 20.6 (9.0) | 11.8 (25.8) | 0.34 (5.8) | 34.7 | 268.2 (2.3) |
| Mushabay | 57.3 (18.5) | 19 (8.8) | 9.25 (18.1) | 0.53 (36.7) | 17.4 | 298 (24.9) |
| Jongar large fruit | 113.6 (14,2) | 12.9 (2.5) | 8.25 (1.8) | 0.84 (4.7) | 9.82 | 38.2 (26.7) |
| Jongar molonimdi | 59.45 (9.6) | 22.8 (1.1) | 10.6 (17.9) | 0.19 (30.7) | 55.7 | 174.6 (16.1) |
| Jongar crimson | 158.5 (3.9) | 21.3 (7.5) | 12.3 (12.9) | 1.08 (23.4) | 11.4 | 137.3 (21.6) |
| Ketpen | 64.13 (19.4) | 27 (9.2) | 11.8 (22.5) | 0.5 (21.9) | 23.6 | 182.8 (1.5) |
| Ile green fruit | 111 (7.68) | 23.37(9.3) | 13.19 (5.2) | 0.32 (5.1) | 41.3 | 46.4 (36.9) |
| Ile honey | 129.3 (5.06) | 23.3 (8.1) | 13.0 (23.8) | 0.57 (13.6) | 22.8 | 49.1 (18.2) |
| ILe | 128.5 (16.5) | 20.5 (6.9) | 13.3 (17.5) | 0.56 (32) | 23.7 | 105.8 (23) |
| Ile medium fruit | 128.27 (14) | 28.8 (9.5) | 13.3 (12) | 0.49 (37.7) | 27.1 | 48.2 (7.8) |
| Average | 95.96 | 20.72 | 11.5 | 0.69 | 21,9 | 147.19 |

*Leucoanthocyanidins*

The antioxidant properties of apple fruits are mainly related to the high content of tannins, more specifically leucoanthocyanidins (Hyson, 2011). Our results showed that the leucoanthocyanidins content in fruits of varieties ranged from 38.2 mg/100 g FW ('Jongar large fruit') to 463 mg/100 g FW ('Usak'). In our cultivars, the average leucoanthocyanidins content was 147.19 mg/100g FW; higher contents ranging from 244.3 to 463 mg/100 g FW were accumulated in fruits of four cultivars. Ten clone varieties (Ketpen, Jongar cider, Jolak alma, Ile medium fruit, Sirga alma, Asya, Askar, Jongar molonimdi, Jongar rootstock, Ile honey) were characterized by high polyphenolic stability (CV from 1.5 % to 18.2 %), but the other clones 'Jongar crimson' 'Jongar autumn fruit', 'Ile', 'Usak', 'Mushabay', 'Jongar large fruit', 'Jongar fir fruit', 'Jongar', 'Jongar kisgilt', 'Ile green fruit' were characterized by high variability in this trait (CV 21.6–36.9%). The content of polyphenols in fruits of 'Jongar large fruit', 'Jongar kisgilt', 'Ile green fruit' and 'Jongar Autumn fruit' varieties significantly differed from the average value for this variety (Table 2).

*Pomological characteristics*

The table 3 data displays the values obtained for various characteristics of fruits such as fruit shape, fruit ground color, fruit overcolor, fruit ribbing, flesh color, flesh firmness, flesh flavor, and fruit stalk length. The pomological description reveals that there is a large variation in fruit shapes and colors among the clone varieties. The fruit shapes observed were globous, globose-conical, flat, flat-globose, oblong-conical, and ellipsoid. The fruit ground color also varied with green-5, light green-2, whitish green-1, yellow-3, light yellow-2, yellow-orange-1, yellow-green-1, greenish and yellow-1, whitish yellow-1, pink-1, purple-1, and purple red-1. The fruit over color showed great diversity with red-4, dark red-4, purple red-3, purple-1, brown red-1, pink-red-2, and none-5. The flavor of wild apples is one of the most significant characteristics. It is known that fruit flavor varies both qualitatively and quantitatively depending on genotype, maturity, and climatic conditions. Furthermore, phytochemicals like sugars, organic acids, and phenolic compounds

contribute to apple flavor. Most of the apple samples had subacid (11) and sweet (6) flesh flavors. However, three clone varieties had astringent (Jolak alma, Jongar cider, Mushabay) flesh flavor.

Table 3: Pomological traits of clone varieties of *M. sieversii*

| Clone varieties | Fruit shape | Fruit ground color | Fruit overcolor | Fruit ribbing | Flesh color | Flesh firmness | Flesh flavor | Fruit stalk length |
|---|---|---|---|---|---|---|---|---|
| Askar | G | LG | R | Absent | Greenish | Medium | Subacid | Short |
| Asya | GC | Pi | PR | Absent | Pinkish | Firm | Subacid | Short |
| Jongar autumn fruit | G | YO | DR | Absent | Yellowish | Firm | Sweet | Medium |
| Jongar rootstock | F | Pu | Abs | Weak | Reddish | Hard | Subacid | Long |
| Jongar kisgilt | FG | G | Pu | Weak | Pinkish | Firm | Subacid | Medium |
| Sirga alma | FG | G and Y | R | Strong | Yellowish | Medium | Subacid | Medium |
| Jongar fir tree | FG | G | R | Strong | White | Soft | Subacid | Medium |
| Usak | G | Y | R | Absent | Yellow | Hard | Subacid | Long |
| Jongar | OC | YG | PR | Weak | Pink | Firm | Subacid | Long |
| Jolak alma | E | LY | BR | Moderate | White | Soft | Subacid | Medium |
| Jongar cider | FG | LY | PuR | Absent | White | Firm | Astringent | Short |
| Mushabay | FG | Y | Abs | Absent | Yellowish | Soft | Astringent | Medium |
| Jongar large fruit | FG | WG | PiR | Strong | Greenish | Firm | Subacid | Medium |
| Jongar molonimdi | G | LG | DR | Absent | Yellow | Soft | Astringent | Medium |
| Jongar crimson | G | PR | Abs | Absent | Reddish | Firm | Subacid | Short |
| Ketpen | G | Y | Abs | Weak | White | Firm | Sweet | Medium |
| Ile green fruit | GC | G | Abs | Absent | Yellowish | Firm | Sweet | Short |
| Ile honey | G | WY | R | Weak | Cream | Hard | Sweet | Short |
| Ile | FG | G | PuR | Absent | Greenish | Firm | Sweet | Short |
| Ile medium fruit | G | G | DR | Weak | Yellowish | Firm | Sweet | Short |

Note: globose-G, F-flat, flat-globose-FG, globose-conical-GC, oblong-conical-OC, ellipsoid-E; Light green-LG, pink-Pi, yellow orange-YO, purple-Pu, green -G, yellow-Y, greenish and yellow-G and Y, yellow green-YG, light yellow-LY, whitish green-WG, purple red-PuR, whitish yellow-WY, Yellow orange-YO, red-R, dark red-DR, absent-Abs, Pink red-PiR, brown red-BR

## Discussion

Twenty evaluated clone varieties of *M. sieversii* selected from Ile Alatau and Zhongar Alatau exhibited high intraspecific diversity in phenological, morphological, and biochemical traits. Traits such as late flowering, attractive appearance, high content of dry matter, sugar, leucoanthocyanidins, and a unique harmonious flavor will be valuable for breeders working on developing trees with high-quality fruits. The fruits of eight evaluated clonal varieties, including Asya, Askar, Jongar large-fruit, Ketpen, Ile green fruit, Ile, Ile honey, and Ile medium fruit, are suitable for fresh consumption. The fruits of varieties Asya, Askar, Jongar large-fruit, Jongar autumn fruit, Jongar, Ketpen, Ile green fruit, Ile, Ile honey, and Ile medium fruit of universal purpose are good raw materials for the production of natural juice. Cider can be prepared from the fruits of Jolak alma, Jongar cider, Mushabay, Jongar, Jongar rootstock, Jongar kisgilt, and Ketpen. Additionally, the clone varieties of *M.sieversii* from the *ex situ* collection are particularly useful for reforestation in degraded areas of the Republic of Kazakhstan due to their adaptation to extreme conditions.

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