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IDENTIFICATION OF VITAMIN C IN FLOWERS AND LEAVES *MALUS* SPP.

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The objective of this study was to evaluate the ascorbic acid content in vegetative and generative organs of crabapple from the collection of the M.M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine. Considering the abundant flowering, higher fruiting in comparison with *Malus domestica* Borkh., increased content of nutrients and biologically active substances in flowers and leaves of crabapple, they deserve wide use in production. The genotypes were planted to a permanent place of growth in 2014. Total ascorbic acid content in flowers ranged from 116 (*Holliana*) to 188 (*Era*) mg / 100 g, in leaves from 112 (*Van Ezeltin*) to 183 (*Royalty*) mg / 100 g.

The objects of the study are valuable for use as raw materials of biologically active additives. Using the flowers and leaves of *Malus* spp., As raw materials for the production of dry powder, will meet the needs of consumers for affordable and nutritious ingredients rich in vitamin C.

Keywords: Malus, crabapple, flower, leaves, vitamin C.

Introduction

One of the most important scientific directions for research today in the introduction and breeding of plants, cultivation, technology, methods of extraction of biologically active substances and creation on their basis of a new generation of food additives demanded for normal life activity of mankind [1, 2].

Vitamin C (Ascorbic Acid) is a water-soluble antioxidant. It was first isolated in 1928, by the Hungarian biochemist and Nobel Prize winner Szent-GyorGyi.

Unlike humans, almost all plants are able to synthesize ascorbic acid because it plays a critical role in photosynthesis [3, 4]. In the process of photosynthesis and further transformations, green plants are able to create any organic compounds necessary for their existence.

Our attention is drawn to the identification of ascorbic acid in the vegetative and generative organs of crabapple [5]. Ascorbic acid is found both in and outside the chloroplasts and in many other tissues where it acts as an antioxidant and free radical scavenger. During stress, drought, UV exposure plants generate free radicals to a much greater extent [6]. During these periods, ascorbate is especially important for the plant because it is the primary defense against oxidative damage. Just as ascorbic acid has many functions in humans, ascorbate plays many roles in plant biochemistry [7]. Almost all plants have mechanisms for ascorbic acid production, and the concentration of this metabolite varies widely [8, 9].

One of the most important functions of ascorbic acid in humans is its role in collagen synthesis. Collagen is a protein that makes up most of the connective tissue in the body, especially in tendons, ligaments and skin [10]. A crucial step in collagen synthesis is the posttranslational hydroxylation of proline and lysine residues, which allows subsequent glycosylation and promotes the formation of a tertiary structure [11]. The enzymes responsible for catalyzing this hydroxylation rely on ascorbic acid as a cofactor. Without ascorbic acid, hydroxylation cannot occur, collagen cannot be produced, and connective tissue weakens, leading to the symptoms of scurvy [12].

Ascorbic acid is also involved in many other biological processes in the human body. It has been found to help in the metabolism of tyrosine, folic acid, and tryptophan, as well as the breakdown of histamine to prevent allergic reactions. Carnitine synthesis and

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many catecholamines, all important biomolecules, also depend on ascorbic acid [13]. Also, ascorbic acid is involved in the metabolism of iron: in the intestine restores Fe^{3+} to Fe^{2+}, promoting its absorption, as well as accelerates the release of iron from ferritin, promotes the conversion of folate into coenzyme forms. With a deficiency of ascorbic acid, the absorption of ferrum is reduced, which causes development of iron deficiency hypochromic anemia [14]. Other nutrients also benefit from the presence of vitamin C. Fat-soluble vitamins such as A, D, E, and K are susceptible to free radical attack, a process that can be reduced if there is vitamin C present to take up an unpaired electron. Other synergistic effects between compounds have been identified, including enhanced regeneration of oxidized vitamin E and ascorbic acid [15].

It has been previously known that in woody plants, it is in the leaves that ascorbic acid is very abundant because they are the main site of photosynthesis in which ascorbic acid plays an important role [16]. And also, plant tissues during growth, such as young leaves, usually contain elevated concentrations of ascorbic acid. This is due to the role of ascorbates in growth processes [15, 16]. Similarly, the minds of most fruits contain higher concentrations of ascorbic acid, much higher than in the pulp due to UV exposure and the resulting need for protection against free radicals.

Studies on the identification of ascorbic acid in *Malus* spp. flower, not enough has been found, so relevant.

Since in many countries the supply of vitamin C is determined by seasonal consumption, it is therefore important also in the off season to provide an adequate supply of food products rich in vitamin C.

Crabapple cheap and highly nutritious little-used fruits with great therapeutic and medicinal value and the use of their vegetative and generative organs as raw materials for the production of dry powder will meet consumer needs for affordable and nutritious ingredients rich in vitamin C.

Material and methods

Plant material. Nineteen crabapples were included in this study. Each specimen was grown for more than 3 years in the same location in the Department of acclimatization of fruit plants of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kiev. All trees were grafted on rootstocks 54-118 in 2014 were planted to a permanent location where they were grown according to standard commercial practices. Studies of vitamin C content in flowers and leaves of crabapple were conducted during the growing season 2021. Flowers were collected during the period of mass flowering, leaves once a month from May to August.

The total content of ascorbic acid

The ascorbic acid content was determined by the titrimetric method, which is based on the addition of an alkaline solution of 2,6-dichlorophenolindophenol (Tilmans reagent) to an acidified solution containing ascorbic acid. While the titrated solution contains ascorbic acid, the added solution of 2,6-dichlorophenolindophenol, which has a deep blue color, discolors with the formation of a leucoform, due to the presence of a reduced form of vitamin C. As soon as the entire amount of ascorbic acid in the analyzed solution is oxidized to dihydroascorbic acid, the solution acquires a red color characteristic of the oxidized form of 2,6-dichlorophenolindophenol in an acidic medium. The results obtained are expressed in mg / 100 g of dry matter (DM) [17].

The mass fraction of ascorbic acid in the analyzed material was calculated by the formula:

$$C_{AA} = \frac{T \cdot V_1 \cdot V_2}{m \cdot V_3}$$

Where T – titer of 0.001 n solution of 2,6-dichlorophenolindophenol by ascorbic acid, 0.088 mg/ml; V_1 – volume of 0.001 n solution of 2,6-dichlorophenolindophenol used for titration of the extract, ml; V_2 – the total volume of the extract, 100 ml; V_3 – the volume of the extract taken for titration; m – the weight of the sample of the test material, g; 100 – conversion factor per 100 g.

Statistical analysis. The results obtained are means ± standard deviation (SD) of three replicates (n=3). Basic statistical analyses were performed using PAST 2.17.

Results and Discussion

Apple plants (*Malus* spp.) Are wild relatives of the apple tree and are becoming increasingly important ornamental resources for germplasm plants worldwide due to their compact crowns and the variety of colorful leaves, flowers and fruits during growth.

The study was aimed at examining the vitamin C content of flowers and leaves. *Malus* spp. Crabapple is simply the name given to any *Malus* species that produces fruit with a diameter of less than 5 cm, many of which exist and are typically named for the area they originated.

Crabapple is most valued from an aesthetic point of view: the color of the foliage, the color of flowers and fruits, abundant and long-lasting flowering and fruiting, the duration of fruit preservation. These characteristics do not exclude or ignore other useful properties, such as: raw materials for the production of vinegar, jams, juices, and then rootstock for example for bonsai. etc., not forgetting about wild animals, especially birds, for which in the winter crabs are one of the main sources of food. Apple fruit or leaf extracts have antioxidant or cytotoxic activity and antimicrobial activity.

As is already well known about the physiological role of ascorbic acid, that it is involved in collagen biosynthesis, is a biological antioxidant and a catalyst of iron ion redox reactions in cytochrome and is very widely used as a nutrient in the pharmaceutical and cosmetic industries and as a preservative because of its antioxidant activity. Ascorbic acid is a powerful antioxidant, plays an important role in the regulation of redox processes, participates in the synthesis of collagen and procollagen (substances involved in maintaining healthy skin tone and prevent varicose veins), folic acid metabolism, folic acid and stem one synthesis, regulates blood coagulation, normalizes capillary permeability required for hematopoiesis, has anti-inflammatory and anti-allergic effects. This vitamin is directly involved in metabolism as coenzymes or constituent components of coenzymes.

Some studies have confirmed that elderly people who take vitamin C together with vitamin E have a 50% lower risk of premature death from disease than people who do not take supplements [18]. California scientists have proven that people who consume more than 750 mg/day of vitamin C reduce their risk of premature death by 60%. [19].

The physiological requirement for vitamin C for an adult is an average of 90 mg per day. This amount is contained in 225 grams of lemon or just 45 grams of black currant. The real need for vitamin C in modern life is much higher than this level, which is why vitaminized foods are so important.

In addition to known crops that have a sufficient supply of vitamin C in leaves such as currants, blackberries and others, ascorbic acid identification studies have been performed on leaves of pumpkin, jute, cubeb pepper, clove basil, curry, African eggplant and moringa. The vitamin C content of the samples ranged from 5.70 to 815.00 mg / 100 ml [20].

Many studies have been conducted on the identification of vitamin c in apple fruit and little attention has been paid to its isolation in color and leaves, so this study is relevant.

The results of the analysis of the content of vitamin C (ascorbic acid) in the analyzed leaves of Malus spp. are shown in Figure 1. Vitamin C content in nineteen samples of fresh crabapple leaves ranged from 100 mg / 100 g (*Adirondack*) to 185 mg / 100 g (*Royalty*).

According to the results of the analysis, it can be argued that the leaves of the genotype *Royalty* apple is promising for use as a raw material for the development of biologically active additives, as well as a donor for the selection of new cultivars with increased ascorbic acid content.

Some sources claim that there is 10 more vitamin C in apple blossoms than in lemons, as well as an abundance of iron and zinc, copper, phosphorus. Apple blossoms have anti-inflammatory and analgesic properties, and tea from the inflorescences is even used to prevent the formation of cancer cells. Infusions of apple blossom are recommended for the treatment of infectious diseases of the gastrointestinal tract, for the prevention of cardiovascular diseases, for lowering blood cholesterol and burning fat.

Conducting a literature review of ascorbic acid content in the flowers of various plants was found that its high content in representatives of the genus *Crataegus* L. ranged from 617.07 mg / 100 g dry weight to 1104 mg / 100 g dry weight, in cultivars of roses cultivated in six different cultivars was 2556, 7323, 9011, 2167, 5045 and 5234 mg / 100 g dry weight [20]. Considering these data, vitamin C identification in crabapple flowers was carried out. Based on the data we obtained in the course of the study it was found that the content of vitamin C in crabapple flowers ranged from 112 mg / 100 g (*Van Ezeltine*) to 183 mg / 100 g (*Royalty*). Thanks to this knowledge in the future will reduce the search for available raw materials for higher ascorbic acid content and for the perspective of breeding work (Fig. 2).

Analysis of Figure 3 shows some dynamics of vitamin C content in vegetative and generative organs of apple-tree plants. It was found that when the ascorbic acid content in flowers is higher, this indicator in leaves is also higher in comparison with other varietal samples. Also, this analysis showed that there is a significant correlation

of ascorbic acid content in the cultivar '*Golden hornet*', which differs by the fact that the flowers have a higher index of vitamin C than the leaves.

Comparative analysis of the content of vitamin C in flowers and leaves gave the following results as a raw material with the highest content of ascorbic acid in flowers marked cultivars: '*Pink paradise*'; '*Pfessor shprengler*'; '*Ola*'; '*Holliana*'; '*Gloriosa*'; '*John*'; '*John Downie*'; '*Kuznetsov paradise*', in leaves: '*Era*'; '*Royalty*'; *Royalty Red*'; '*Van Ezeltine*'.

The results of studying the identification of vitamin C in various apple cultivars are important for the theoretical substantiation of breeding directions based on the identification of promising crabapple phenotypes (Fig. 4).

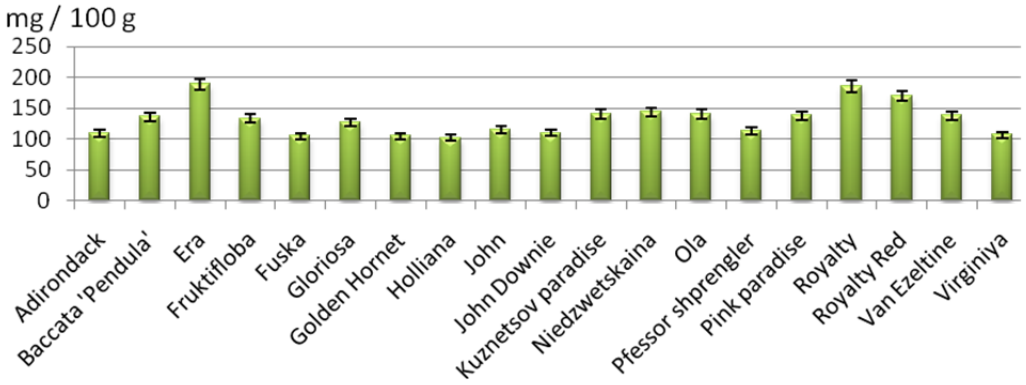

Fig. 1. Vitamin C content in leaves of *Malus* spp. genotypes

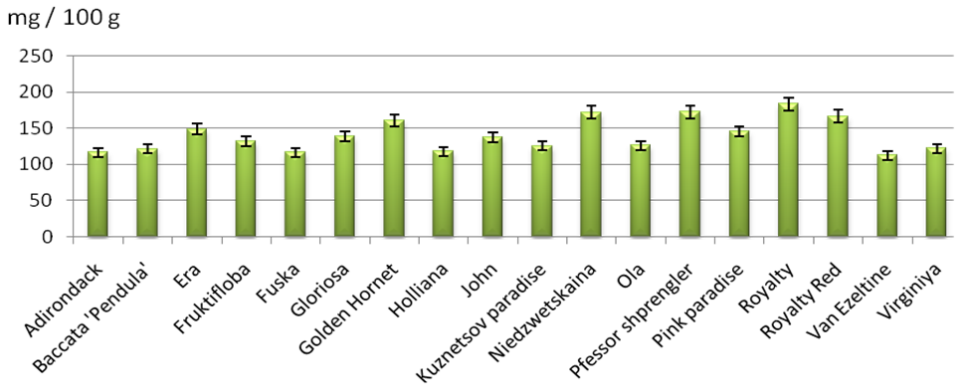

Fig. 2. Vitamin C content in flowers of *Malus* spp. genotypes

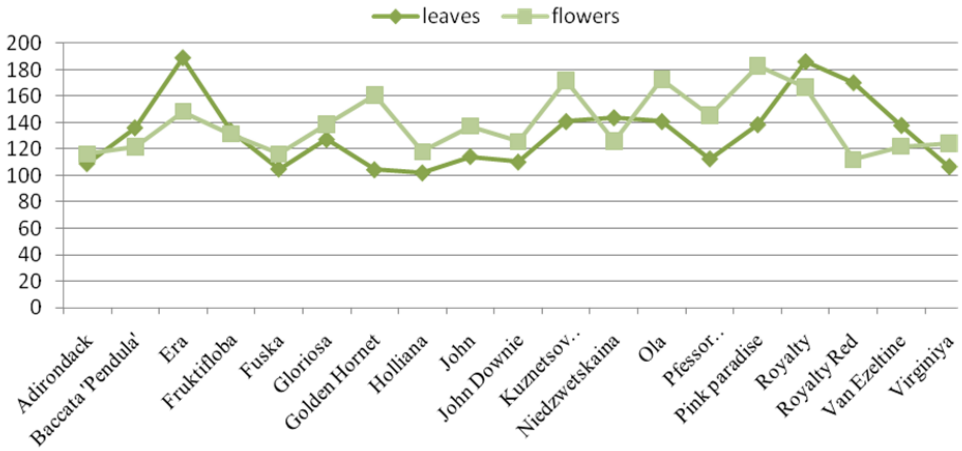

Fig. 3. Comparative content of vitamin C in flowers and leaves of *Malus* spp.

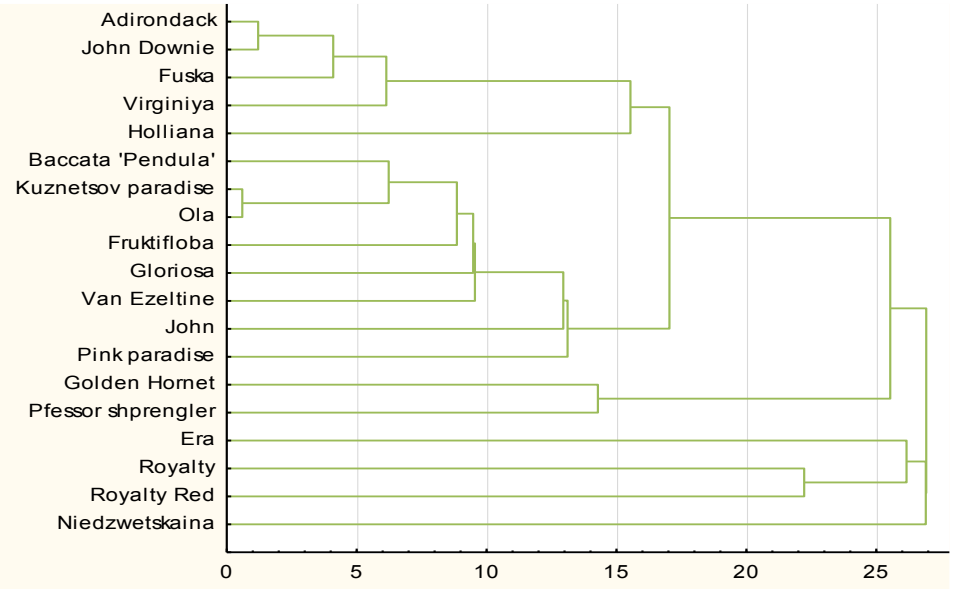

Fig. 4. Dendrogram of 19 genotypes of *Malus* spp. based on the content of ascorbic acid in leaves and flowers

The dendrogram was constructed using the method of hierarchical cluster analysis using the average to distinguish similar groups among various initial data on the content of ascorbic acid in leaves and flowers. Figure 3 clearly shows significant differences between the tested genotypes of *Malus* spp. In this study, 19 genotypes were grouped into two main clusters based on the greatest similarity. The most closely related were (*Ola, Kuznetsov paradise*), the similarity from other genotypes of the collection both in the color of leaves and flowers and in the content of ascorbic acid. Cluster II contained two related (*Adirondack, John Downie*) genotypes of *Malus* spp. The apple tree (*Niedzwetskyana*) is separated into a separate cluster, taking into account its species origin and the creation on its basis of many other representatives of this genus.

Availability of raw materials, namely different genotypes of crabapple, the possibility of its cultivation, unpretentiousness to the conditions of habitat and the possibility of harvesting in large quantities allows you to use the objects of research not only in the production of juices, jams but also in pharmaceutical products, and in other industries.

Conclusions

The aim of the study was to identify the best cultivars from the 19 phenotypes of *Malus* spp. collection of M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, according to the content of vitamin C in flowers and leaves to select promising, which can be used in breeding programs to improve the biochemical composition and as a raw material base for biologically active additives.

Given the importance of these underutilized plants, the study attempted to estimate the vitamin C content of future food products for the benefit of society, and the results of this study could potentially be used in the production of medicinal substances with widespread use in the pharmaceutical and natural cosmetics industries.

As a raw material with the highest content of ascorbic acid in flowers marked cultivars: '*Pink paradise*'; '*Pfessor shprengler*'; *'Ola'; 'Holliana'; 'Gloriosa'; 'John'; 'John Downie'; 'Kuznetsov paradise'*, in leaves: '*Era'; 'Royalty'; Royalty Red'; 'Van Ezeltine'*. The dynamics of the maximum accumulation of vitamin C in leaves during the growing season was observed in May during the development of the leaf lamina and in July during the effect of high temperatures.

As well as the results of the study showed that the *R'oyalty', 'Era'* and '*Niedzwetskyana'* crabapple are very promising for detailed breeding work, for creating new cultivars with an increased content of vitamin C in the vegetative and generative organs of the plant.

Overall, the present study shows that plants of the genus *Malus* spp. Due to the availability of raw materials, the possibility of cultivation and a sufficient content of vitamin C, they are promising for the creation of effective medicines and other products that are in demand in various industries.

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