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Some mechanisms of winter resistance in apricot flower buds in the period of ecodormancy

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Abstract. The accelerated development of flower buds during the thaw in apricots and almonds during the ecodormancy period leads to significant damage to the flower elements during return frosts and loss of future crops. The aim of the research was to identify the mechanisms of delay in the rate of development of flower buds during the ecodormancy period, their relationship with the degree of frost resistance and the timing of flowering in apricots. The following indicators of flower buds were analyzed: the degree of exit from endodormancy, frost resistance at temperatures of -18 °C and -31 °C, the degree of morphological development of flower elements, the activity of α-amylases at temperatures of + 15 °C and + 60 °C, total content water, phenolcarboxylic acids, flavonoids and free proline. A significant positive correlation was revealed between the percentage of death of flower buds at a temperature of -18 °C and the percentage of buds emerging from endogenous dormancy (0.64*), the percentage of death of buds at a temperature of -31 °C and the degree of development of flower elements (0.70*), water content and the degree of development of flower elements (0.76**), amylase activity at +60 °C and amylase activity at +15 °C (0.76**), the content of phenolcarboxylic acids in the bark of shoots and flower buds (0.61*). For the first time, psychrophilic forms of α-amylases have been discovered in apricot flower buds.

Key words: frost resistance of flower buds, psychrophilic α-amylase, thermal stability of α-amylases, α-amylase inhibitors, phenolic compounds.

INTRODUCTION

Winter hardiness of flower buds is the main problem of stone fruit crops in the northern hemisphere (Yablonskiy & Markovich, 1970a; Sholokhov & Savvina, 1975). The greatest winter hardiness of flower buds corresponds to the endodormancy period, as buds break from endodormancy their winter hardiness is also lost (Glozer, 2010). The mechanism of endodormancy has not yet been fully understood, but it is known that its duration depends on both the genotype (Olukolu, 2010) and the number of low positive temperatures in the autumn-winter period (Viti et al., 2010). There is also evidence that plant break endodormancy can be produced not only by low positive temperatures, but

also by respiration inhibitors (Walton et al., 2009; Hegazi, 2012; El Masri et al., 2018), producers of active oxygen radicals, or strong oxidants (Bailly et al., 2008; Sarath & Mitchell, 2008). Without break endodormancy, the plant is incapable of further development. This problem arises in hot countries, where, for some cultivars with long-term dormancy, there is a lack of low positive temperatures to exit endodormancy (Rouse et al., 2006). If a plant approaches the growing season with incomplete exit from dormancy, then it develops very slowly and, as a rule, drops flower buds (Alburquerque et al., 2003). During endodormancy, both hydrolytic and synthetic processes are blocked (Seif El-Yazal & Seif El-Yazal, 2013), no new gene products capable of breaking dormancy arise (Gumilevskaya & Azarkovich, 2007; Dogramaci et al., 2010), but allowed oxidative polymerization of reserve substances - carbohydrates (Cooke et al., 2012; Fadon et al., 2018), fatty acids (Porta & Rocha-Sosa, 2002), phenolic compounds (Ji et al., 2015). After break endodormancy, hydrolytic processes are gradually activated. One of the first events in cells after break endodormancy is the hydrolysis of a very hydrophilic protein, dehydrin (Yamane et al., 2006).

Hydrolysis of 1,3-β–glucan (callose) in the plasmodesmata and sieve tube (lat.*tuboli cribrosi*) of the vascular bundles, after break endodormancy, opens the way for tissue watering (Leubner-Metzger & Meins, 2001; Leubner-Metzger, 2003; Cilia & Jackson, 2004) and creating conditions for the activity of other hydrolases. Then starch (Fadon et al., 2018), oligosaccharides (Yablonskiy & Markovich, 1970), and lipids (Seif El-Yazal & Seif El-Yazal, 2013) are gradually hydrolyzed. In different apricot varieties, the endodormancy period can vary from 300 chill units for varieties - 'A.1740' and 'Gold Kist' (Bradley & Maurer, 2002; Olukolu et al., 2009), to 1,266 and 1,450 chill units for varieties - 'Orangered'® and 'Zard' (Kostina, 1969; Guerriero et al., 2002).

Modern apricot varieties, in a temperate climate zone, lack endodormancy for the entire frosty period, which reduces their winter hardiness. In Central Russia, the maximum duration of endodormancy (up to the 1^{st}–2^{nd} decade of February) in such apricot varieties as: 'Zavodskoy No. 1', 'Saratov ruby'® (Golubev et al., 2020), 'Manitoba 604' (Licznar–Małańczuk & Sosna, 2005), but even their rest is not enough for resistance to thaws. Most of the varieties of Central Russia come out of dormancy by mid-January. After the endodormancy is over, the sum of positive temperatures (Razavi et al., 2011) and the maximum values of thaw temperatures are of greatest importance for the development of flower buds. In the more southern regions of the country, where there are many thaws in the second half of winter and the ambient temperature can reach +15 – +20 °C, internal factors that restrain the rapid development of flower buds are of particular importance. Studies show that the rate of development of flower buds during the ecodormancy period and their winter hardiness depend on the sensitivity of the genotype to the photoperiod (Stirling et al., 2002; Avdeev, 2014), the time of differentiation of flower buds (Tuz, 1960; Nemeth et al., 2008), the rate of development elements of the flower (Yablonskiy, 1970), the intensity of the breakdown of carbohydrates, indirectly indicating the activity of hydrolytic enzymes (Yablonskiy & Markovich, 1970; Seif El-Yazal & Seif El-Yazal, 2013), the content of phenolic inhibitors (Morozova, 1970; Korableva, 1974; Nenko et al., 2018; Paliy et al., 2018), sensitivity to thaw temperatures (Genkel & Oknina, 1964), biosynthesis of stress proteins (Levit et al., 1990). During the ecodormancy period, flower buds develop especially rapidly in almonds (Amygdalus communis L.) (Prudencio et al., 2020) and apricots (Prunus Armeniaca L.) (Tuz, 1960). Our research is aimed at identifying the

mechanisms of delaying the rate of development of flower buds during the ecodormancy period, their relationship with the degree of frost resistance and flowering periods, as well as the classification of apricot genotypes according to the mechanisms of protection against premature awakening of flower buds, which will help combine several resistances in the created variety and prevent them death from recurrent frosts.

MATERIALS AND METHODS

The material for the research was a collection of apricot varieties and hybrid forms (h.f.) of the private breeding nursery Golubevs, Saratov (Table 1).

Table 1. Characteristics of the studied samples

Genotype	Origin	Delayed or acceleration flowering
'Zavodskoy No. 1'	local form	very late, +3 days
'LXVI–09–1'	'XV-03-1' x 'Late blooming №4'	very late, +3 days
'7589'	*P. brigantiaca* x *P. armeniaca* 'Makhtobi'	very late, +3 days
Plum 'Svetlana'	unknown	very late, +3 days
'VII–05–1'	Plum 'Svetlana' x apricot h.f. 'Original'	very late, +3 days
'Late blooming EM'	local form	very late, +3 days
'Saratov ruby' ®	sibirian h.f. 'Handsome' x h.f. 'Pharaon'	middle (23.04.2020)
'Manitoba 604'	'Scout' x 'MeClure'	early-middle, -0.5 days
'Zhigulevsky souvenir'	unknown	early, -1 day
'Gonsi Magyar kajszi'	'Magyar Kajszi' clone	early, -1 day
'Generous'	h. f. 'Pharaoh' x h.f. of Baikalov	very early, -2 days
'*P. sibirica* No. 1'	unknown	superearly, -3 days

The analyzed plants grow in one area, without differences in microrelief, soil fertility and microclimate. Breeding forms - 'Zavodskoy No. 1', 'LXVI-09-1', 'VII-05-1', 'Saratov ruby' ®, 'Generous', '*P. sibirica* No. 1' grow on their roots, genotypes - '7589', 'Late blooming EM', 'Manitoba 604', 'Zhigulevsky souvenir', 'Gonsi Magiyar kaiszi' are grafted onto the same seminal rootstocks of apricot, the eastern plum 'Svetlana' is grafted onto a seedling home plum (*Prunus domestica*). Samples were taken from the southeastern side from the middle part of the crown, simultaneously for all analyzes (except for determining the release of flower buds from dormancy) from the same trees at the age of 12 years.

The winter hardiness of fruit buds was determined in mid-February (February 16, 2020) (the third component of winter hardiness is frost resistance during thaws), after holding for 24 hours at temperatures of -18 °C or -31 °C (rapid cooling) and the 3rd days at a temperature of +25 °C. The winter hardiness of fruit buds was analyzed in 2 replicates, 100 buds each. The number of dead buds was counted on longitudinal sections under an MBS-10 binocular microscope and expressed as a percentage of the total number of buds.

The stages of morphophysiological development of apricot flower buds were studied under an MBS-10 binocular microscope with a Levenhuk M300 BASE Microscope digital camera. The degree of development of flower elements was assessed using 12 photographs of longitudinal sections of flower buds of each cultivar for each sampling date (12/18/2019, 01/22/2020, 02/16/2020). The delay or acceleration of

flowering was assessed visually by the difference in the dates of mass flowering (more than 80% of open flowers) in the studied genotypes in comparison with the apricot cultivar 'Saratov Ruby'® with an average flowering period (04/23/2020).

The degree of interruption of dormancy of flower buds in the studied genotypes was determined by the percentage of flowers capable of blooming after 15 days on cut branches placed in water at +25 °C, guided by Richardson's method (Richardson et al. 1974). For analysis, three branches of each variety were cut off (01/22/2020) with at least 120–150 flower buds each. The water was changed every 3 days. All flower buds capable of developing into a flower to the white bud stage were classified as dormancy breaking.

The α-amylase activity was determined using 3,5-dinitrosalicylic acid (DNSA) (Miller, 1959). The flower buds stored at -20 °C were ground in a porcelain mortar with the addition of quartz sand. The extract for analysis was obtained as follows: 0.5 g of crushed flower buds were added with 5 mL of phosphate buffer (pH 6.8), and then incubated for 1 hour at 4 °C with constant shaking. Then the samples were centrifuged at 10,000 rpm for 10 minutes. Further, all parameters were determined in the supernatant fluid.

The calibration graph was built for maltose, in triplicate.

Substrate for fermentolysis: 15 mL of phosphate buffer (pH 6.8) and 5 mL of 0.9% sodium chloride solution were heated to 80–90 °C, a suspension consisting of soluble starch 0.5 g and 20 mL of distilled water was added, boiled for 1.5–2 minutes, brought to 50 mL, and then cooled.

Enzymatic hydrolysis was carried out as follows: 1 mL of the extract (supernatant) was incubated with 1 mL of the substrate at 60 °C for 120 minutes or at 15 °C for 24 hours. To determine the amylase activity, 0.1 mL of the enzyme mixture was taken, 0.3 mL of 3,5-dinitrosalicylic acid reagent was added to it and incubated for 10 minutes at 100 °C in a water bath. Then 3 mL of distilled water cooled to +4 °C was added.

The optical density (D) of the colored solution was determined on a spectrophotometer Model - 752N 190–960 nm (China) at a wavelength of 530 nm. Calculation of α-amylase activity was performed using a calibration curve. The a-amylase activity was expressed in mg of maltose released per minute per 100 mg wet weight of the sample. The determination was carried out in three biological and three analytical replicates.

Phenolic compounds were extracted three times from the bark of annual apricot shoots or flower buds with 70% ethanol at 80 °C in a flask with a reflux condenser. The extraction was carried out as follows: 300 mg of plant material dried at 110 °C (flower buds or bark of annual shoots) was poured for the first time with 10 mL of 70% ethanol and heated in a water bath (80 °C) for 30 minutes. It was re-extracted with 10 mL of 70% ethanol under the same conditions for 20 minutes. The third time was extracted with 5 mL of 70% ethanol for 10 min. The extracted material and extracts were combined, transferred quantitatively to centrifuge tubes, and centrifuged at 6,000 rpm for 10 minutes. The supernatant was made up to 25 mL with 70% ethanol.

The study was carried out in 3 biological and 3 analytical replicates. The aliquot for analysis was 0.2 mL.

The total content of phenolcarboxylic acids was determined by the method developed for plant raw materials (Petukhova & Mirovich, 2019), spectrophotometrically at a wavelength of 325 nm. For the quantitative determination of flavonoids, we used the method (Methods for assessing..., 2012), based on the ability of flavonoids to form a stable complex with a citrate-boric reagent with an absorption maximum at 420 nm.

The content of flavonoids and phenolcarboxylic acids (PCA) in flower buds or bark of annual shoots on February 16, 2020 in apricot, plum, and plum-apricot hybrids was expressed in mg per 1 g of dry weight. The calibration graph for determining the concentration of phenolcarboxylic acids was built using gallic acid, and for flavonoids, using quercetin.

Thin layer chromatography of flavonoids was carried out in an upward flow of solvents toluene - acetone - formic acid at a ratio of 40: 30: 6 or on an OPTLC 'Chrompress-25' instrument using plates for HPTLC / OPTLC Silica Gel Pre-coated Aluminum Sheets.

The proline content in flower buds was determined by colorimetric or spectrophotometric methods at λ 530 nm, in glass cuvettes, using a ninhydrin reagent and sulfosalicylic acid (Bates et al., 1973). The concentration of proline in the samples was expressed in mg per 1 g of dry weight of the sample. Proline was determined in 3 biological and 5 analytical replicates. The weighed amount was 200 mg.

The water content in flower buds was determined by the gravimetric method, after drying to constant weight in a thermostat at 110 °C. For the analysis, a sample of 1 g of plant material was taken in 3 replicates.

All experimental data were processed statistically using the AGROS program, version 2.09.

RESULTS AND DISCUSSION

Freezing of apricot branches with flower buds in mid-February during a thaw at a temperature of -18 °C showed the distribution of genotypes according to the degree of damage, from 8.20% to 91.07% (Table 2).

Table 2. The degree of frost resistance of flower buds, their release from dormancy and the timing of flowering

Genotype	Percent death at temperature, as of 16.02.2020		Percent of flower buds recovery from dormancy on 01/22/2020	Degree of develop-ment of flower elements	Water content in flower buds (%)
	-18 °C	-31 °C			
'Zavodskoy No. 1'	8.20	100.00	8.77	6	52.12
'LXVI–09–1'	9.06	100.00	10.68	5	53.93
'7589'	12.05	97.78	14.48	2	44.30
Plum 'Svetlana'	15.19	46.00	41.88	1	48.46
'VII–05–1'	20.01	95.18	33.62	3	48.37
'Late blooming EM'	25.00	99.00	49.61	4	46.73
'Saratov ruby' ®	30.11	97.02	49.15	4	47.72
'Manitoba 604'	34.50	100.00	83.36	6	55.81
'Zhigulevsky souvenir'	19.17	100.00	69.52	5	54.03
'Gonci Magyar Kajszi'	74.54	100.00	54.64	6	52.07
'Generous'	91.07	100.00	89.09	6	55.54
'*P. sibirica* No. 1'	19.61	100.00	67.75	6	51.11
LSD 0.05	17.672	7.033	21.244		2.513

There is a tendency to an increase in the death of flower buds with an increase in the percentage of their exit from endodormancy (Correlation coefficient 0.64*). The available feature correlations are shown in Table 3.

The Table 2 shows that in all very late flowering genotypes, the death of buds at -18 °C does not exceed 25%, whereas in early ('Gonsi Magyar kaiszi') and very early ('Generous') flowering genotypes, death can reach 74.54 and 91.07%, respectively. Late blooming genotypes differed quite strongly in the percentage of flower buds coming out of dormancy - from 8.77% to 49.61%. Late flowering genotypes differed even more in the degree of development of flower elements. Fig. 1 shows the degree of development of floral elements in the most contrasting genotypes.

Table 3. Correlation analysis between frost resistance and some other characteristics of flower buds

Correlation coefficient matrix					
	1	2	3	4	5
1	1.00				
2	0.20	1.00			
3	0.64*	0.10	1.00		
4	0.43	0.70*	0.41	1.00	
5	0.41	0.27	0.50	0.76**	1.00

* Significant at 5% level; ** Significant at 1% level; 1 – percent death of flower buds at -18 °C; 2 – percent death of flower buds at -31 °C; 3 – percentage of awakened buds; 4 – degree of development of flower elements; 5 – Water content in flower buds, %.

In our study, all genotypes could be divided into 6 groups according to the degree of development of flower elements (Table 2, Fig. 1). The first degree of development of flower elements was characterized by small, poorly formed anthers with a high degree of transparency, a small transparent pistil, transparent petals, and the presence of free space in the bud. From the first to the sixth degree, there was an increase in the size of the bud as a whole and all the elements of the flower, the formation of their typical structure, a decrease in transparency and free space. The sixth degree of bud development was characterized by well-formed all structural elements of the flower with large, opaque anthers, a large pistil with a rounded basal part and a formed stigma.

A particularly strong lag in the morphophysiological development of flower buds was observed in the eastern plum 'Svetlana' and a distant hybrid '7589' of the alpine plum and apricot (*P. brigantiaca x P. armeniaca* 'Makhtobi'). It is known from the literature that the degree of winter hardiness of flower buds depends on the stage of morphophysiological development; the more advanced the morphophysiological stage of flower bud, the lower its hardiness (Sholokhov & Savvina, 1975). Flower buds of varieties and hybrids created on the basis of Siberian genotypes ('Manitoba 604', 'Generous', '*P. sibirica* No. 1') are characterized by an accelerated rate of morphophysiological development of flower buds (5 и 6 degree of development of flower elements). Flower buds after emerging from endogenous dormancy, in order to maintain viability at critical temperatures (in our case, -31 °C), require additional protective mechanisms aimed at curbing the development of floral elements and the accumulation of water content in the buds. The very low correlation coefficient between the percentage of flower bud deaths at -31 °C and the percentage of awakened flower buds (0.1) indicates that not all late flowering genotypes have mechanisms to inhibit the rapid rate of bud development during the ecodormancy period. When flower buds are exposed to a critically low temperature of -31 °C during the ecodormancy period, it is not the degree of dormancy or even the flowering period of the genotype that comes to the fore, but the stage of morphophysiological development of flower buds (correlation

coefficient 0.70*) and the water content in cells. In very late flowering genotypes ‘Zavodskoy No. 1’and ‘LXVI-09-1’) with the lowest percentage of buds coming out of dormancy (8.77 and 10.68%, respectively), but with more advanced stages of development of floral elements (6 and 5, respectively), at extremely low temperatures (-31 °C), the death of flower buds was 100%. The highest (statistically significant) frost resistance (death of only 46%) was shown by the oriental plum variety ‘Svetlana’, in which the average percentage of recovery from dormancy of flower buds (41.88%), but the greatest lag in the development of the structural elements of the flower (underdeveloped anthers, unformed stigma pistil; see Table 2 and Fig. 1). The total water content in the buds of more than 50% was critical for all genotypes, regardless of the timing of flowering.

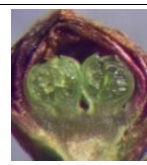
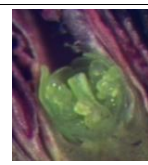
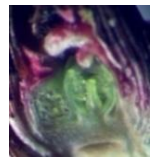
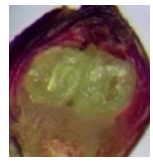
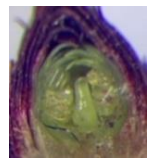
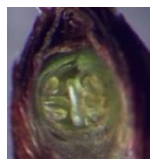
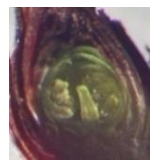
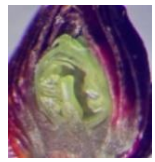
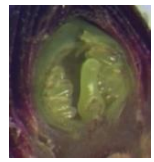
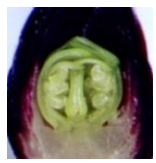
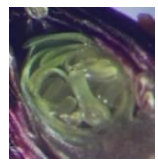
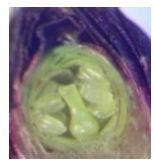
Variety, hybrid, shape	Flower buds morphology on: 12/18/2019	01/22/2020	02/16/2020	Degree of development of flower elements
Plum ‘Svetlana’				1
‘7589’				2
‘VII–05–1’				3
‘Late blooming EM’				4
‘LXVI–09–1’				5
‘Manitoba 604’				6

Figure 1. The degree of development of floral elements in the most contrasting genotypes at different periods of rest.

The correlation between the degree of development of flower elements and water content is statistically significant and amounts to 0.76** at the 1% significance level. All genotypes with earlier flowering than the apricot variety 'Saratov Ruby' had a more developed morphophysiological stage of flower buds (well-formed, opaque, anthers; pistil with a rounded base and a formed stigma), increased water content (more than 50%) and 100% flower buds death after exposure to temperatures of -31 °C. It is possible that active protection mechanisms are more important at critical temperatures, for example, osmoprotectors (Arora et al., 1996; Arora R. 1996; Liu et al., 2007; Szabados & Savoure, 2009; Chen et al., 2012), antifreeze proteins (Griffith et al., 1992; Yeh et al. 2000; Griffith et al., 2005; Provesi et al., 2016), cold shock proteins (Perras & Sarhan, 1989; Gong et al., 2002; Nakaminami et al., 2009; Takahashi et al. 2013) and chaperones (Samuel et al., 2000; Karlson & Imai, 2003; Piszczek et al., 2005; Shimosaka & Ozawa, 2015).

So, studies have shown that a delay in the stage of morphophysiological development of flower buds is an essential adaptive mechanism in increasing winter hardiness.

To identify the reasons for the accelerated rate of development of apricot flower buds during the ecodormancy, the activity of α-amylase, as one of the thermolabile enzymes, was studied. Consider the existing prerequisites for such research. During the endodormancy period, the maximum accumulation of starch was found in flower buds (Fadon et al., 2018), and as it emerges from dormancy, its amount decreases. The enzyme hydrolyzing native starch is α-amylase (Manners, 1974). An important role of α-amylase in plant life is that, with the participation of this enzyme, such a storage organic matter as starch is converted from a non-transportable form into transport sugars, heading to growth points. Other amylolytic enzymes are also involved in the degradation of starch, but the contribution of α-amylase is to initiate this process. Only α-amylase is able to break down intact starch granules (Manners, 1974). Apricot, in contrast to plum, is considered a "starchy" breed, that is, in its tissues, part of the starch accumulated in the autumn period remains throughout the entire endodormancy period (Bosieva & Nartikoeva, 2014). In addition, an increase in amylase activity largely characterizes the intensity of the processes of 'physiological swelling' associated with the accumulation of osmotically active substances (Obrucheva & Antipova, 1997). Some researchers (Carginale et al., 2004) associate damage to flower buds in apricot during thaws with the presence of its own temperature threshold in each genotype - the threshold of sensitivity to positive temperatures. When warming, varieties with a low temperature threshold react quickly, initiating biochemical reactions leading to the hydrolysis of reserve nutrients and the formation of substances that promote growth. Cultivars with a high temperature threshold require more heat to initiate similar reactions. There is evidence (Wagner et al. 2017) that the developmental processes of the male gametophyte in plants blooming in winter and early spring are able to adapt to low temperatures during evolution.

To study the activity of α-amylase at contrasting temperatures, genotypes of all flowering periods were selected - from super early to very late, different sensitivity to thaws and frost resistance of flower buds. The experiments were carried out on 9 apricot genotypes, 2 apricot-plum hybrids and one oriental plum variety (Table 4). Amylase activity was tested at 2 temperatures - +60 °C and + 15 °C, and the enzymatic lysis at 60 °C was carried out for 120 minutes, and at 15 °C - during the day.

Table 4. Activity of α-amylase, (mg of maltose released per minute per 100 mg of wet weight of the sample), content of flavonoids (mg per 1 g of dry buds), the content of phenol carboxylic acids (PCA) (mg per 1 g of dry buds) and proline (mg per 1 g wet weight of flower buds) in February buds (16.02.2020) apricot, plum and plum-apricot hybrids

Genotype	α-amylase activity in flower buds, at		Flavonoid content in flower buds	Content of PCA		Proline content in flower buds
	60 °C	15 °C		in the shoot bark	in flower buds	
'Zavodskoy No. 1'	0.71	0.02	34.56	6.82	11.87	1.05
LXVI–09–1	0.87	0.76	41.92	7.28	12.91	1.25
'7589'	1.38	0.09	2.57	11.85	16.39	1.46
Plum Svetlana	0.37	0.15	41.99	9.54	12.68	1.21
VII–05–1	1.05	0.17	26.17	6.91	13.01	1.06
'Late blooming EM'	2.39	0.19	38.20	9.51	12.79	2.01
'Saratov ruby'®	1.19	0.26	35.23	7.58	9.68	0.68
'Manitoba 604'	0.80	0.84	37.76	9.23	12.79	0.70
'Zhigulevsky souvenir'	0.74	0.03	10.01	7.25	9.43	1.12
'Gonsi Magyar kaiszi '	0.74	0.06	39.49	5.90	10.63	1.12
'Generous'	1.48	0.90	32.79	7.73	9.23	1.21
P. sibirica no 1	4.04	2.22	5.53	7.98	7.5	0.93
LSD 0.05	0.637	0.092	3.320	0.619	1.039	0.063

Studies have shown that genotypes rapidly awakening in the thaw, with an advanced stage of morphophysiological development of flower buds ('Manitoba 604', 'Generous', '*P. sibirica* No. 1'), created on the basis of Siberian forms, have α-amylase activity at low temperatures (15 °C) was several times higher than in genotypes with delayed development of flower buds ('Saratov Ruby', 'Svetlana' plum, 'VII-05-1', 'Late blooming EM') (Fig. 2).

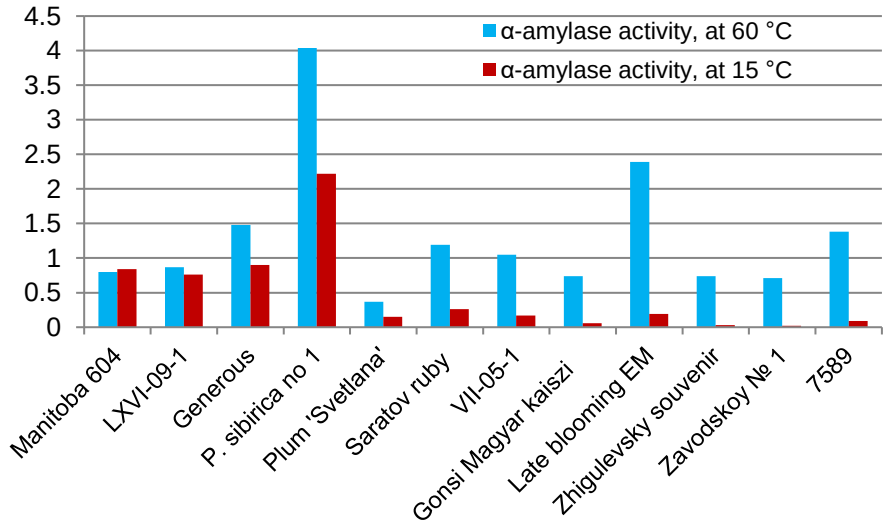


Figure 2. The activity of α-amylase (the amount of formed maltose (mg) per minute per 100 mg of raw buds) at different temperatures.

The graph shows that genotypes created with the participation of Siberian forms (‘*P. sibirica* No. 1’, ‘Generous’, ‘Manitoba 604’) have increased α-amylase activity both at high incubation temperatures (60 °C) and at low temperatures (15 °C). From which it can be concluded that in the genotypes ‘*P. sibirica* No. 1’, ‘Manitoba 604’ and’ Generous’ hydrolytic enzyme α-amylase is more adapted to low temperatures and is able to start working already in thaws, which entails hydrolysis of starch, saturation of tissues with water, more intensive development of flower buds and, as a result, a decrease in their frost resistance. It is known from the literature (Gianese et al., 2001; D’Amico et al., 2001; D’Amico et al., 2002) that is sufficient a point mutation in the structural gene of α-amylase, leading to the replacement of only one amino acid in protein molecule so that this enzyme becomes cold-resistant and begins to work at lower temperatures. With the Siberian forms of apricot, similar mutations apparently occurred and psychrophilic isoforms of α-amylases were formed. After exposure to low-temperature refrigeration (-31 °C) on flower buds of genotypes with high amylase activity at + 15 °C - ‘*P. sibirica* No. 1’ (2.22 mg min^{-1} per 100 mg wet weight), ‘Manitoba 604’ (0.84), ‘Generous’ (0.90), ‘LXVI - 09-1’ (0.76) mortality was 100%, and in genotypes with low enzyme activity - plum ‘Svetlana’ (0.15), VII-05-1 (0.17), ‘Saratov Ruby’ (0.26), mortality was 46%, 95.18% and 97.02%, respectively. Thus, the temperature sensitivity of α-amylase is an important indicator in the characterization of the genotype - the physiological activity of its flower buds during the thaw. Measurement of α-amylase activity at different temperatures can serve as a kind of marker, with the help of which it will be possible to isolate genotypes that are least sensitive to thaws and have the highest winter hardiness flower buds.

Based on the literature data (Sholokhov & Savvina, 1975) that varieties with the same rate of morphogenesis when crossing do not always give the same picture of inheritance of this trait in the offspring, it can be concluded that there are several mechanisms of restraining the development of flower buds. Phenolic compounds may be one of the factors regulating the rate of morphogenesis. The maximum accumulation of phenolic compounds in the scales of flower buds is observed in the autumn-winter period, and at the time of blooming, their number is minimal (Zhang et al., 2020). Phenolic compounds are powerful antioxidants and inhibitors, both in plants and in other organisms. The group of phenolic compounds is not homogeneous in their biological action, some of them are Indoleacetic acid (IAA) oxidase inhibitors (phenolcarboxylic acids: vanillic acid, ferulic acid; flavonoids: luteolin, quercetin, etc.), the other part, on the contrary, are IAA oxidase stimulants (phenolcarboxylic acids: p-coumaric acid, p-hydroxybenzoic acid; flavonoids: apigenin, kaempferol, etc.) (Volynets, 2013). A number of articles (Wang et al., 2010; Chen et al., 2013; Li et al., 2014; Yuan et al., 2014) showed the inhibitory activity of phenolic compounds, primarily flavonoids, on α-amylases and α-glycosidases human. We assume that the premature awakening of flower buds depends on hydrolytic enzymes - α-amylases, α-glycosidases, β-glucosidases, 1,3-β–glucanases. The presence of inhibitors of these enzymes in flower buds or bark of annual shoots will increase the winter hardiness of genotypes. So, when studying the effect of biologically active substances on the activity of alpha-amylase and frost resistance of winter wheat (Atimoshoae & Titika, 1990), it turned out that substances that reduce the level of enzyme activity during the period of hardening of plants increase their frost resistance. A more significant decline in alpha-amylase activity corresponds to a higher frost resistance.

We studied two groups of phenolic compounds - phenol carboxylic acids (PCA) and flavonoids, in flower buds (in mid-February) and bark (in late October) in apricot, plum and their hybrids. Studies have shown (Table 4) that the greatest amount of PCA in the bark of annual shoots accumulates in the genotypes with the latest flowering - hybrid *'P. brigantiaca* x *P. armeniaca* Makhtobi '(11.85 mg g^{-1} dry weight of bark), 'Late blooming EM' (9.51), Plum 'Svetlana' (9.54). The 'Manitoba 604' cultivar (9.23 mg) has a slightly lower, but rather high PCA content in bark, which, although it does not have a flowering delay, showed restrained amylase activity at 60 °C. The highest content of PCA in flower buds is observed in late flowering genotypes: 'Late blooming EM' (18.36 mg g^{-1} d.w.), '*P. brigantiaca* x *P. armeniaca* Makhtobi '(16.39 mg),' VII-05-1 '(13.01 mg),' Late blooming LXVI-09-1 '(12.91 mg), early flowering forms showed a significantly lower content - '*P. sibirica* No. 1' (7.5 mg), 'Generous' (9.23 mg), that is, the same trend has remained. A positive correlation (0.61*) was found between the PCA content in bark and flower buds (Table 4).

Table 4. Correlation analysis of flower buds α-amylase activity and its potential inhibitors

Correlation coefficient matrix								
	1	2	3	4	5	6	7	8
1	1.00							
2	0.20	1.00						
3	-0.03	0.30	1.00					
4	0.09	0.19	0.76**	1.00				
5	0.25	-0.27	-0.48	-0.28	1.00			
6	-0.31	-0.28	0.15	-0.01	-0.31	1.00		
7	-0.40	-0.18	-0.41	-0.52	0.08	0.61*	1.00	
8	-0.07	-0.05	0.17	-0.28	0.00	0.39	0.41	1.00

* Significant at 5% level; ** Significant at 1% level; 1 – % death of flower buds at -18 °C; 2 – % death of flower buds at -31 °C; 3 – amylase activity at 60 °C; 4 – amylase activity at 15 °C; 5 – flavonoids in flower buds; 6 – PCA in the bark of shoots; 7 – PCA in flower buds; 8 – proline in flower buds.

Since each genotype has its own set of PCA, which can act in different directions, it is difficult to find the dependence of the α-amylase activity on the total PCA. Correlation analysis still showed, albeit a small (mathematically insignificant) negative relationship between the total PCA content in flower buds and α-amylase activity both at 60 °C (-0.41) and at 15 °C (-0.52). The component analysis of phenol carboxylic acids should provide more convincing data. A similar pattern was observed in the total content of flavonoids. The highest content of flavonoids was found in late blooming genotypes - 'Svetlana' plum (41.99 mg g^{-1} of dry weight of buds), 'LXVI-09-1' (41.92) and 'Late blooming EM' (38.20). In early flowering forms, for example, '*P. sibirica* No. 1', the content of flavonoids was only 5.53 mg g^{-1} of dry weight of buds, in another early flowering variety 'Zhigulyovskiy souvenir' there were 10.01 mg of them. It is interesting to trace the relationship between the sum of flavonoids and the activity of α-amylase. There is a slight inverse relationship (correlation coefficient -0.48) between the α-amylase activity and the accumulation of flavonoids: the more there are, the lower the α-amylase activity for most genotypes. It should also be noted that not all flavonoids and their glycosides inhibit α-amylases with the same potency (Wang et al., 2010; Chen et al., 2013; Li et al., 2014; Yuan et al., 2014).

Preliminary data from chromatographic analysis of flavonoids on silica gel plates show that the number of components in different genotypes varies from 2 to 10. For the time being, 3 flavonoids have been identified - rutin, naringenin and quercetin. Research in this direction continues.

The next line of research is to test the role of free proline in the delay of flowering and the formation of frost resistance of stone fruit buds. There are data in the literature on osmoregulation and competitive inhibition of hydrolases by L-proline - in the struggle for free water (Kiyosue et al., 1996; Nakashima et al., 1998). The analysis showed that the highest proline concentrations in mid-February accumulated flower buds of late flowering genotypes - 'Late blooming EM' (2.01 mg g^{-1} flower buds wet weight) and *'P. brigantiaca* x *P. armeniaca* Makhtobi '(1.46), and the smallest ones are 'Saratov ruby' (0.68), 'Manitoba 604' (0.70) and *P. sibirica* No.1 (0.93). There is a weak (insignificant) inverse dependence of the α-amylase activity on the accumulation of free proline - the more the hydrophilic amino acid itself, the lower the enzyme activity (correlation coefficient -0.28).

Multiple mechanisms of flowering delay and protection from low temperatures complicate the understanding of the role of one or another component, but comprehensive studies will make it possible to isolate genotypes with one mechanism or another, combine them in one genotype, and create flower buds with high winter hardiness. For breeding purposes, the most valuable genotypes should combine a low dormancy rate of flower buds, low free water content, low α-amylase activity during thaws, and high accumulation of proline and flavonoids. Out of the studied samples, only 2 genotypes correspond to these characteristics - 'Svetlana' plum and 'Late blooming EM' apricot, which will be used in further breeding work.

CONCLUSIONS

The study showed the complex nature of the formation of not only the winter hardiness trait of apricot flower buds, but also the timing of flowering of genotypes. All studied genotypes were characterized by the flowering period, the degree of recovery from dormancy, the rate of morphogenesis of flower buds, their frost resistance, cold resistance of α-amylases, the accumulation of phenol carboxylic acids, flavonoids and proline, and water content.

As a result of this study, the following genotypes valuable for breeding were identified that showed the least flower buds death at -18 °C: 'Zavodskoy No. 1' (8.2%), 'LXVI–09–1' (9.06%), '*P. brigantiaca* x *P. armeniaca* Makhtobi' (12.05%), 'Svetlana' plum (15.19%), 'VII-05-1' (20.01%). The genotype with the highest winter hardiness of flower buds at a critical temperature of -18 °C was revealed - 'Svetlana' plum.

Correlations were revealed between the frost resistance of apricot flower buds at a temperature of -18 °C and the percentage of buds break from endodormancy (0.64*), the percentage of flower buds death at a temperature of -31 °C and the degree of development of flower elements (0.70*), water content and the degree of development of flower elements (0.76**).

For the first time, psychrophilic forms of α-amylases were found in apricot flower buds. Genotypes with adaptation of α-amylases to low temperatures - *P. sibirica* No. 1' and 'Generous', 'Manitoba 604', 'LXVI-09-1' are needed for further comparative studies.

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