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LOW TEMPERATURE TOLERANCE OF APPLE CULTIVARS OF DIFFERENT PLOIDY AT DIFFERENT TIMES OF THE WINTER

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Artificial freezing was used to evaluate diploid and triploid apple cultivars from the All Russian Research Institute of Fruit Crop Breeding at Orel throughout three winters. The studied apple varieties were developed by breeder E. N. Sedov and cytological analysis was carried out by cytologist G. A. Sedysheva. In early winter, all cultivars exhibited high tolerance to cold. In mid-winter buds and wood were severely damaged, while bark was more resistant for most cultivars. Basic components of hardiness were estimated: component I — frost resistance at –25 °C in the beginning of winter; component II — maximum value of frost resistance at –40 °C developed by plants during hardening; component III — ability to retain the hardened condition at –25 °C after a period of three-day thaw at +2 °C; and component IV — the ability to restore frost resistance at –30 °C after repeated hardening and three-day thaw at +2 °C. During late-winter thaws, buds suffered from frosts, while the bark and wood retained frost hardiness. Late in winter all cultivars demonstrated high resistance to repeated frosts. Triploid cultivars exhibited the highest level of cold hardiness of vegetative buds, bark and wood of annual shoots throughout the winter; these cultivars included ‘Zhilinskoye’, ‘Vavilovskoye’, ‘Osipovskoye’, ‘Patriot’, ‘Sinap Orlovski’, ‘Spasskoye’, ‘Turgenevskoye’, and diploids ‘Bolotovskoye’, ‘Sokovinka’, and ‘’Ranneye Aloye’.

Key words: *apple, diploid, triploid, winter resistance, artificial freezing.*

INTRODUCTION

Accelerated evaluation of initial material for winter hardiness is an important stage in apple breeding. At present, laboratory freezing is commonly used to evaluate plant winter hardiness. In the middle zone of Russia, cold injury can occur at different times of the winter. During early winter, cold events of –25 °C may occur and during mid-winter minimum temperatures of –40 °C occur once in forty years. Thaws of 2 °C followed by a freeze of –25 °C in February as well as repeated freezes of –30 °C after thaw at 2 °C and repeated hardening in March can injure plants (Turina *et al.*, 2002).

The halt of shoot growth and initiation of dormancy are important conditions for seasonal apple adaptation to unfavourable winter factors. Under natural conditions the autumn reconstructions in apple plant metabolism begins with gradual decline of air temperature and shorter light days. While entering dormancy, plants stop growing, protective substances are accumulated, cell structures and features change and they enter the hardening phases. Damages to fruit plants by early winter frosts occur by death of cambium cells and bark of shoots. Severe damages to bark and cambium occurs when these tissues have not yet passed the processes of hardening (Saveliev *et al.*, 2010). An important factor for apple cultivation in Russia is maximum winter hardiness, which occurs during hardening till the middle of winter. In the middle of winter, bark and cambium damage is rarely observed in hardened plants. The reason for this is that during the adaptation to negative temperatures, cambium and bark cells are dehydrated by increase of bound water, which protects them against the formation of intracellular ice. Wood is mostly open to injury in the hardened state. The frost resistance mechanism of wood is limited because some water flows out of parenchymal cells during frosts, while the remained water is not able to flow out due to the resistance of cell walls; this results in wood damage (Kichina, 1999). When the temperature becomes higher for three or more days in winter, the physiological condition of trees changes and their resistance to frost decreases. Thus in nature, slight freezing or death of apple trees is observed as a consequence of sharp temperature declines during thaws in February, which cause apple trees to break deep dormancy.

The adaptability of apple cultivars provides plant resistance in the thaw period. Buds, bark and cambium consisting of active metabolic cells are more damaged than wood during thaw periods. It is very important for plants in late winter to retain hardening to low temperature after thaw. Thaws in March are dangerous for apple plants since they are in the exogenous dormancy and higher temperatures activate bud growth processes, while gradual temperature decline after a thaw favours the restoration of cambium and bark resistance. Buds are less able to restore hardening state after prolonged thaws. Wood responds less to thaws and does not lose frost resistance during thaws (Turina, 2000). Resistance of apple cultivars to stress in winter is continually being studied due to its practical importance. The development of cultivars that have resistance to negative temperatures in their genotypes is one of the leading directions in fruit breeding.

Cold tolerance at different times of the winter has been studied for apple (Linden, 2002; Czynczyk *et al*., 2004; Ozherelieva *et al*., 2011; Cline *et al*., 2012; Ozherelieva and Sedov, 2014), raspberry (Ozherelieva and Bogomolova, 2011), sour and sweet cherry (Chmir, 2003), cherry plum and apricot (Szalay *et al*., 2010), and sea buckthorn (Ozherelieva *et al*., 2016). The aim of the present study was to evaluate cold resistance of diploid and triploid apple cultivars from the All-Russian Fruit Crops Breeding Research Institute (VNIISPK) during winter using artificial freezing and to select winter hardy cultivars. We estimated potential resistance of apple cultivars to low temperature stressors during winter.

MATERIALS AND METHODS

The investigations were performed in 2012–2014 in the Laboratory of Fruit Plant Resistance Physiology at the VNIISPK. Apple cultivars of different ploidy were studied. The studied diploid and triploid apple varieties were developed under the leadership of the Russian Academy of Sciences academician Professor E. N. Sedov. The cytological analysis of apple variety ploidy was carried out in the cytoembryology laboratory at the VNIISPK by the Doctor of agricultural sciences G. A. Sedysheva (Sedov *et al*., 2008).

The cultivars were grafted on seedling rootstock. The trees were 20 years old. Three typical trees were taken as samples from each cultivar. Annual shoots of moderate length (20–30 cm) were used. They were cut from different sides of trees, five shoots per freezing temperature. The material was taken in late November when the average daily air temperature stabilised below 0 °C for all dates of freezing. The material was kept in a deep freezer at –4 ± 1 °C in plastic bags. Maximal and minimal temperatures of autumn-winter periods during the years of studies are given in the Table 1.

Artificial freezing was conducted in a climate chamber "Espec" PSL-2KPH (Japan) by the method of Turina *et al.* (2002). The basic components of hardiness were studied: component I — frost resistance in the beginning of winter; component II — maximum frost resistance developed by plants during hardening; component III — ability to retain hardening after a thaw period; component IV — ability to restore frost resistance after repeated hardening and thaw (Turina *et al*., 2002). To evaluate the acclimatisation ability of apple cultivars in early winter, shoots were frozen at –25 °C for 8 hours on December 7 (Component I). To evaluate the resistance of apple cultivars to maximally low temperature in mid-winter the shoots were frozen at –40 °C for 8 hours on January 15 (Component II). To evaluate the ability to retain resistance during thaws in winter the shots were frozen at –25 °C for 8 hours on February 10, after a three-day thaw at +2 °C (Component III). To evaluate resistance to repeated frosts in late winter the shoots were frozen at –30 °C for 8 hours on March 20 after a three-day thaw +2 °C and repeated hardening at –5 °C for 5 days, and +10 °C for 5 days (Component IV). The temperature was lowered at a rate of –5 °C per hour.

Cold injury was evaluated by growing shoots in vessels with water. After each freezing, 2–3-cm segments of annual shoots were cut and placed in vessels with water at +21 ± 1 °C for 10 days. The water was changed each two days.

The degree of bud damage was evaluated on a scale of 0 to 5; where 0 = no damage; 1 = insignificant damage, the mesophyll under the bud was damaged; 2 = reversible damage, a part of the leaf bud was damaged; 3 = moderate damage, vascular system and most of leaf buds were damaged; 4 = severe damage, apical meristems and most of leaf buds were dead; and 5 = buds and tissues were dead.

Bark and wood (xylem) damage was evaluated by extent of tissue browning on longitudinal and cross-sections according to the following scale: 0 = no damage, tissues were light coloured; 1 = insignificant damage, 10–12% of tissue area was brown; 2 = reversible damage, 20–40% of tissue area was brown; 3 = moderate damage, 40–60% of tissue area was brown; 4 = severe damage, 60–80% of tissue area was brown and 5 = ?80% of tissue area was dead.

The experimental data were analysed by analysis of variance and means for each date of freezing and were compared by Least Significant Difference methods at the 5% level of significance. Winter hardy 'Antonovka Obykno-

Table 1

MAXIMUM AND MINIMUM TEMPERATURES OF AUTUMN–WINTER PERIODS DURING THREE YEARS OF STUDY

Temp., °C	2012			2013						2014					
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Oct.	Nov.	Dec.
Max	22.5	11.5	11.5	0.8	2.5	5.3	16.5	13.5	2.5	7.8	4.0	19.0	20.8	11.5	5.2
Min	–4.8	–8.0	–27.8	–31.7	–20.0	–22.0	–8.0	–7.5	–19.3	–31.0	–31.0	–10.6	–15.2	–19.8	–23.3

vennaya' and moderately winter hardy 'Melba' diploid cultivars were used as controls.

The experimental data were analysed using mathematical statistics (Dospehov, 1985) using MS Excel software.

RESULTS

Exposure to –25 °C early in December showed that all of the studied apple cultivars demonstrated high cold resistance. Buds, bark and wood of annual shoots were not damaged by –25 °C in early winter during the years of study.

There were significant differences ($p < 0.05$) between cultivars in mid-winter low temperature resistance of buds ($LSD_{05} = 2.3$), bark ($LSD_{05} = 1.0$) and wood ($LSD_{05} = 2.2$). At –40 °C in January the diploid apple cultivars 'Bolotovskoye', 'Ranneye Speloye', 'Sokovinka' and the triploid cultivars 'Zhilinskoye', 'Vavilovskoye', 'Osipovskoye', 'Patriot', 'Sinap Orlovsky', 'Spasskoye' and 'Turgenevskoye' had the same level of cold resistance as 'Antonovka Obyknovennaya'. Reversible damage to buds, bark and wood of annual shoots with scored less than or equal to 2.0 were recorded for many cultivars. The triploid cultivars 'Aleksandr Boiko', 'Maslovskoye', 'Orlovski Partizan', and 'Prazdnichnoye' showed bark and bud injury similar to 'Antonovka Obyknovennaya' (injury rating ≤ 2.0) and bark injury was similar to that of 'Melba' (2.2–2.4 rating).

Significant differences among cultivar were observed for bud ($LSD_{05} = 0.7$), bark ($LSD_{05} = 0.7$), and wood ($LSD_{05} = 0.8$) injury in winter following a thaw. Following exposure to –25 °C after a three-day thaw at +2 °C in February, the triploid apple cultivar 'Spasskoye' was resistant to de-acclimation during the thaw period; there was insignificant damage to vegetative buds, bark and wood. The other genotypes showed a level of cold resistance similar to the control cultivars, where reversible damage of buds, bark and wood were observed.

In March there were significant cultivar differences for bud ($LSD_{05} = 0.4$), bark ($LSD_{05} = 0.4$) and wood ($LSD_{05} = 0.6$) injury following a thaw and repeated hardening**.** After a thaw at +2 °C and repeated hardening, all of the cultivars were able to regain resistance to recurrent freezing at –30 °C. In late winter, the damage observed for vegetative buds (≤ 2.0 rating), and also bark and wood (≤ 1.0) was reversible (Table 2).

The general winter hardiness of cultivars is categorised in Table 3 by ploidy. Cultivars with bud, bark and wood damage ratings of 1.0 to 2.0 rating were included in the winter hardy group, whereas cultivars with damage ratings of 2.1 to 3.0 rating were considered moderately hardy (Table 3).

Diploid and triploid apple cultivars from the VNIISPK breeding programme, listed in Table 3, were evaluated.

DISCUSSION

Results from artificial freezing allowed to identify apple genotypes with high cold resistance of vegetative buds and shoot tissues in early winter due to early cold acclimation. In mid-winter, buds and wood were cold damaged more severely; bark was more resistant for most genotypes, as hardiness was lost slower than for bud or wood tissue. During thaw periods, vegetative buds suffered more cold injury because they de-acclimated, while the basic tissues maintained cold resistance. Late in winter all of the studied apple genotypes showed high resistance of buds and tissues to cold following winter thaws owing to their lack of de-acclimation.

The following winter hardy triploid apple cultivars were identified: 'Zhilinskoye', 'Vavilovskoye', 'Osipovskoye', 'Patriot', 'Spasskoye', 'Turgenevskoye', and 'Sinap Orlovski'. Hardy diploid cultivars included 'Bolotovskoye', 'Ranneye Aloye', and 'Sokovinka'. All of these genotypes possessed hardiness throughout winter at the same level as that of 'Antonovka Obyknovennaya'. ANOVA showed intervarietal differences. Significant relationships occurred between temperature and the degree of bud damage (r = –0.62), bark damage (r = –0.76) and wood damage (r = –0.88) in the apple varieties. A significant relationship between temperatures and ploidy of the varieties was not revealed.

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Table 2

ARTIFICIAL APPLE FREEZING RESULTS (YEARS 2012–2014)

Cultivar	7 Dec. -25 °C			15 Jan. -40 °C			10 Feb. +2°,-25 °C			20 Mar. +2°,-5°, -10 °C, -30 °C		
	Buds	Bark	Wood	Buds	Bark	Wood	Buds	Bark	Wood	Buds	Bark	Wood
Antonovka Obyknovennaya (control)	0[z]	0[y]	0	0.9	0.7	1.3	1.0	0.6	0.5	0.7	0.2	0.4
Melba (control)	0	0	0	2.4	1.6	2.2	2.0	1.2	1.2	1.3	0.6	0.7
Triploid cultivars												
Osipovskoye	0	0	0	1.8	1.0	1.6	1.5	0.9	0.5	0.8	0.3	0.4
Turgenevskoye	0	0	0	1.7	0.7	1.9	1.8	0.7	0.9	1.4	0.3	0.6
Vavilovskoye	0	0	0	1.5	0.5	1.7	1.5	0.0	0.1	1.8	0.4	0.5
Prazdnichnoye	0	0	0	1.5	0.4	2.1	1.2	0.4	0.4	1.8	0.8	1.1
Aleksandr Boiko	0	0	0	2.0	0.6	2.2	2.0	0.6	0.7	1.8	0.2	0.7
Spasskoye	0	0	0	1.9	0.7	2.0	0.6	0.1	0.2	1,2	0.6	0.6
Zhilinskoye	0	0	0	1.5	0.8	1.4	1.3	0.6	0.3	1.5	0.5	0.6
Orlovsky Partizan		0	0	2.0	1.6	2.3	1.4	0.7	0.4	1.0	0.4	0.7
Maslovskoye	0	0	0	2.0	0.9	2.2	1.1	0.2	0.2	1.6	0.8	0.5
Ministr Kisilev	0	0	0	2.1	1.0	2.3	2.0	2.0	0.5	1.9	0.6	0.4
Patriot	0	0	0	2.0	1.0	2.0	1.5	1.5	0.6	1.0	0.3	0.6
Sinap Orlovsky	0	0	0	1.5	1.3	1.5	1.5	1.5	0.5	1.0	0.5	0.5
Diploid cultivars												
Bolotovskoye	0	0	0	1.4	1.1	1.2	1.4	0.6	0.5	0.5	0.4	0.4
Ranneye Aloye	0	0	0	1.7	1.0	1.4	1.5	0.5	0.7	0.7	0.3	0.9
Orlovsky Pioner	0	0	0	2.0	2.0	2.0	2.1	2.0	1.9	2.0	1.5	1.5
Sokovinka	0	0	0	1.7	0.9	1.6	1.7	0.4	0.8	1.5	0.5	0.6
Podarok Uchitelu	0	0	0	2.3	1.0	2.2	2.0	0.6	0.6	1.5	0.4	0.3
Ivanovskoye	0	0	0	2.5	2.3	2.0	1.8	1.2	1.0	2.0	1.8	1.7
LSD$_{05}$				0.5	0.9	0.6	0.7	0.7	0.8	0.4	0.4	0.6

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[z] Bud injury was rated as 0 = no browning; 1 = insignificant injury, the mesophyll under the bud was damaged; 2 = reversible injury, part of the leaf bud was brown; 3 = moderate injury, vascular system and most of leaf buds were injured; 4 = severe injury, apical meristems and most of leaf buds were dead; and 5 = buds were dead.

[y] Bark and wood were rated as 0 = no injury, tissues were light-coloured; 1 = insignificant damage, 10–12% of tissue area was brown; 2 = reversible damage, 20–40% of tissue area was brown; 3 = moderate damage, 40–60% of tissue area was brown; 4 = severe damage, 60–80% of tissue area was brown and 5 = over 80% of tissue area was dead.

Table 3

RELATIVE COLD HARDINESS CLASSIFICATION OF 20 APPLE CULTIVARS BASED ON CONTROLLED FREEZING TESTS CONDUCTED OVER THREE YEARS

Genotype (origin)	Group of hardiness
Antonovka Obyknovennaya (control) *diploid*	Winter hardy
Melba (control) *diploid*	Moderately winter hardy
Triploid cultivars	
Vavilovskoye (29-21-168)[18-53-22(Skryzhapel × OR18T13 × Wealthy Tetraploid]	Winter hardy
Zhilinskoye (Redfree × Papirovka Tetraploid)	Winter hardy
Osipovskoye (Mantet × Papirovka Tetraploid)	Winter hardy
Patriot [16-37-63(Antonovka Krasnobochka × SR0523) × 13-6-106 (Suvorovetz Seedling)	Winter hardy
Sinap Orlovski (Sinap Severny × Pamyat Michurina)	Winter hardy
Spasskoye (Redfree × Papirovka tetraploid)	Winter hardy
Turgenevskoye [18-53-22 (Skryzhapel × OR18T13) × Wealthy Tetraploid]	Winter hardy
Prazdnichnoye (Prima × Giant Spy)	Moderately winter hardy
Aleksandr Boiko (Prima × Wealthy Tetraploid)	Moderately winter hardy
Orlovski Partizan (Orlik × 13-6-106 (Suvorovetz Seedling)	Moderately winter hardy
Maslovskoye (Redfree × Papirovka Tetraploid)	Moderately winter hardy
Ministr Kisilev (Chistotel × Wealthy Tetraploid)	Moderately winter hardy
Diploid cultivars	
Bolotovskoye (Skryzhapel × 1924)	Winter hardy
Ranneye Aloye (Melba × Papirovka)	Winter hardy
Sokovinka (Antonovka Krasnobochka × SR0523)	Winter hardy
Ivanovskoye (Wealthy × Prima)	Moderately winter hardy
Orlovski Pioner (Antonovka Krasnobochka × SR0523)	Moderately winter hardy
Podarok Uchitelu (Karavella × Orlovim)	Moderately winter hardy

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DAŽĀDAS PLOIDITĀTES ĀBEĻU ŠĶIRŅU TOLERANCE PRET ZEMĀM TEMPERATŪRĀM DAŽĀDOS ZIEMAS PERIODOS

Pētītas diploīdās un triploīdās ābeļu šķirnes, kuras izveidotas selekcionāra Jevgeņija Sedova (Krievija) vadībā. Lielākie zemo temperatūru izraisītie bojājumi konstatēti ziemas vidus periodā. Lielākajai daļai šķirņu pumpuri un koksne ir jūtīgāki pret zemām temperatūrām nekā miza. Triploīdās šķirnes uzrāda augstāku aukstumizturību, salīdzinot ar diploīdām šķirnēm.