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ORIGINAL ARTICLE

Cold Hardiness of Apple and Changes in Dehydrin Composition

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The work was aimed to study the degree of wood damage through artificial freezing and dehydrin changes in apple bark. The relation between apple tree cold hardiness and dehydrin accumulation and degradation rate was established.

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Low negative temperature is a major limiting factor for expanding the area of growing *Malus* variety representatives in northern areas. Plant selection breeders obtained quite a large amount of winter-resistant varieties, however, field tests do not provide a fast method of determining reliability of a certain variety under concrete conditions. Laboratory modeling of stress temperature conditions helps accelerate the process of selecting more resistant forms.

The primary damaging factor is cold-induced dehydration (dewatering) of tissue resulting in the loss of functional structure. In addition to other protective mechanisms (accumulation of sugars, pralines, betaine), plant cell accumulates highly hydrophilic thermostable proteins called dehydrins (Close, 1997).

The present work is aimed to find out whether dehydrins composition changes throughout cold period of the year and whether these changes are related to cold resistance of various apple trees genotypes under artificial freezing.

MATERIALS AND METHODS

Six apple trees genotypes were used as an object of the study: Siberian or Berry Apple tree (*Malus baccata*) and Domestic or Cultivated Apple tree (*Malus domestica*) of Papirovka variety (folk selection), as well as hybrids of cultivated and berry apple trees of various crossing generations: Purpurovaya crab apples (unknown origin), semi-cultivated apple trees Veselovka (selection of SB RAS Central Botanical Garden), Krasa Buryatii (selection of Buryat Fruit and Berry station), Altai Ruddy (selection of SRI of Siberian Horticulture

named after M.A. Lisavenko) (Pomology..., 2005; Pomology: Apple tree..., 2005). Contrasted winter-resistance of the above apple trees genotypes was used as a criterion for selecting research objects. Siberian Berry apple tree was used as a stock tree. The studies were conducted in 2008-2013 on the basis of Siberian Institute of Plant Physiology and Biochemistry, SB RAS, Irkutsk.

Apple trees varieties were assessed by winter resistance parameters under artificial freezing (Program and methods..., 2005). The degree of tissue damage in cut branches was determined by acquisition of brown color in degrees from 0 to 5. To provide freezing temperature we used a low-temperature chamber with the negative temperatures range of -10 to -80°C. Thawing conditions (+5°C) were modeled in a thermostat produced by Sanyo. Freezing time equaled 8 - 24 hours.

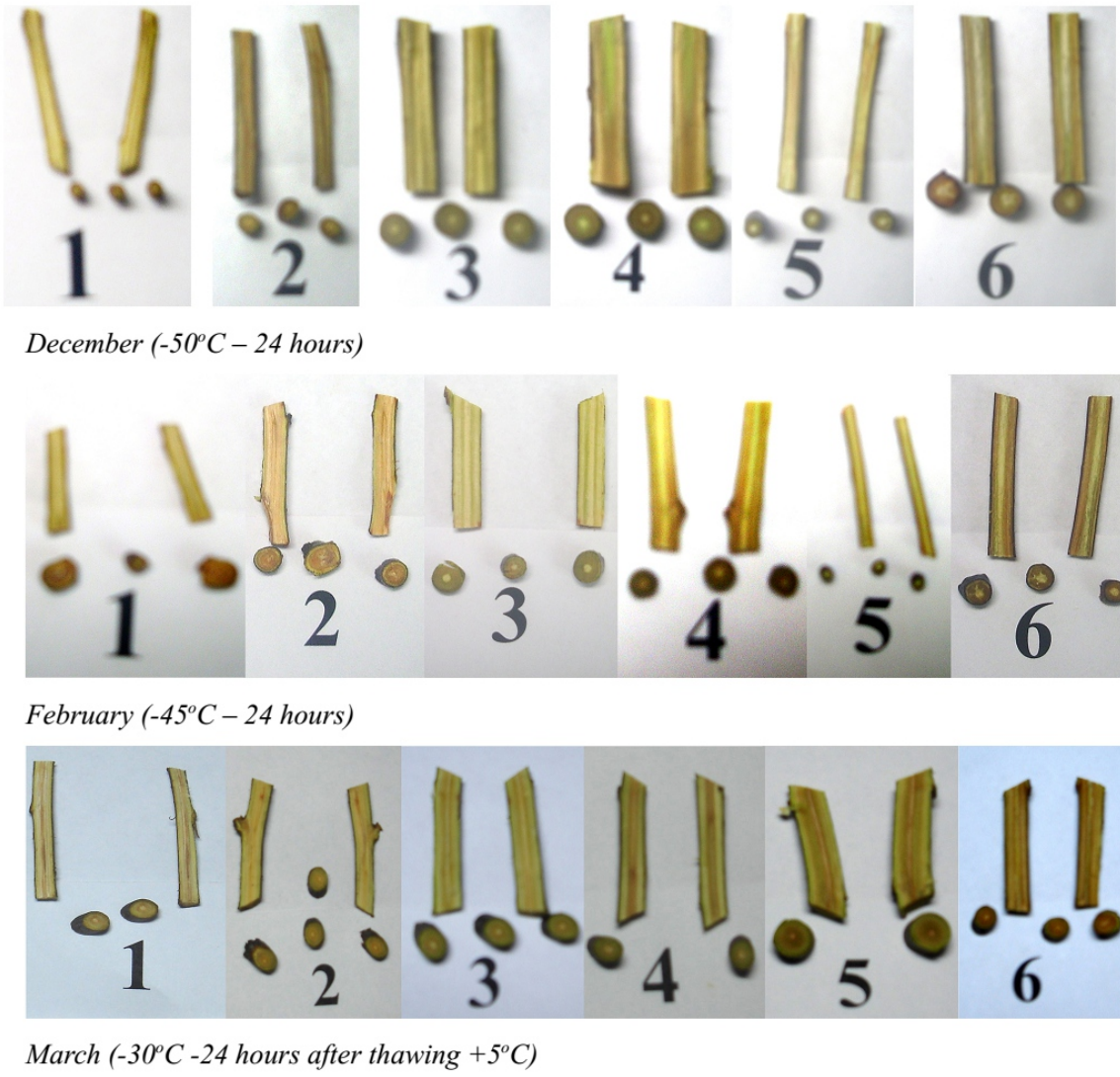

Figure 1 Wood damage (brown color intensity) in various apple trees varieties under artificial freezing: 1 – Siberian Berry apple tree; 2 – crab apple Purpurovaya; 3 – Veselovka; 4 – Krasa Buryatii; 5 – Altai Ruddy; 6 — Papirovka.

Current year branches collected from the plants growing in uniform agrotechnical and climatic conditions. The bark was removed from the branches and frozen in liquid nitrogen. The samples

were kept in a freezer at -80 °C until protein extraction. Total protein was extracted according to the standard method adopted for arboreal plants (Arora *et al.*, 1992). Protein concentration was determined as per Lowry's method [Lowry *et al.*, 1951). Following protein separation with Na-DDS-electrophoresis in 14% PAAG, immunoblotting with antibodies for dehydrins was performed (Agrisera AS07 206) (Timmons, Dunbar, 1990).

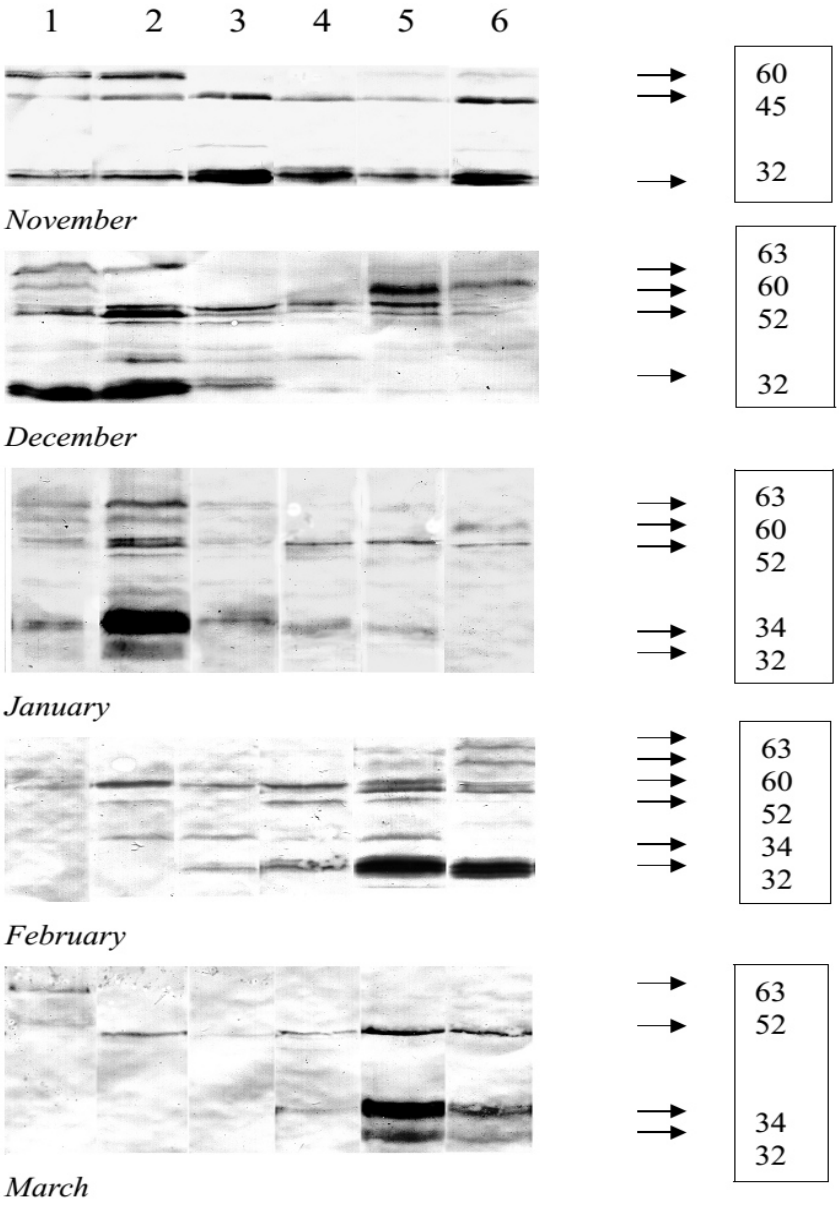

Figure 2. Changes in dehydrins content of apple tree bark from November till March: 1- Berry apple tree; 2- crab apple Purpurovaya; 3 – semi-cultivated apple tree Veselovka; 4 - semi-cultivated apple tree Krasa Buryatii; 5 - semi-cultivated apple tree Altai Ruddy; 6 –cultivated apple tree Belyi Naliv.

RESULTS AND DISCUSSION

Freezing was carried out in three stages: December (at the temperature range from -30 to -50°C), February (at the temperature range from -35 to -45°C), March (at the temperature -40°C and through the thawing period at +5°C to -30°C). The first stage freezing did not reveal any damage of the wood of the genotypes under study. In February

insignificant browning (2 points) was observed only in Papirovka. In March a complete death was observed in Papirovka (5 points) after -40°C and significant damage in Altai Ruddy (4 points) at -30°C after thawing period (Fig. 1).

The immunoblotting data analysis revealed significant differences in the rate of dehydrins accumulation and degradation in the bark of various apple trees genotypes for the whole period of study (Fig. 2).

Already in November-January the range of dehydrins of genotypes differing in resistance showed quantitative and qualitative difference. If Siberian apple tree, crab apple Purpurovaya, semi-cultivated apple trees Veselovka and Krasa Buryatii demonstrated intense process of dehydrins accumulation, Altai Ruddy and Papirovka showed no increase in dehydrins amount. During this time proteins with molecular weights of 63, 60, 52, 34 and 32 kDa were determined. Changes in dehydrins composition took place in February. The amount of these proteins in Berry apple tree, crab apple, Veselovka and Krasa Buryatii considerably drops as compared to Papirovka and Altai Ruddy. In March the bark of Berry apple tree and crab apple contain virtually no dehydrins, but this fact doe not reduce resistance of these genotypes neither at -40°C nor at -30°C after thawing +5°C. Changes in dehydrins range towards increase in Altai Ruddy and Papirovka in February-March did not contribute to better withstanding of extreme temperature impact.

CONCLUSION

The tests conducted lead to the assumption that the higher the rate of dehydrins accumulation and degradation, the higher the apple tree cold resistance. The study of changes in dehydrins composition of fruit trees is of undoubted interest for modern researchers of not only plant physiology, but also field workers of agriculture. This kind of information would facilitate correction of the selection process towards higher winter resistance and accelerate introduction of new varieties in the regions with unstable climate.

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