Winter-hardiness assessment in ground-covering roses by histochemistry

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Abstract. The paper presents the results of the seasonal dynamics of starch and lipids study in annual shoots of rose species and cultivars growing in the urbanized medium of a Siberian mega-polis under continental climate conditions. The prospects of prediction the winter hardiness of roses by the rate of starch hydrolysis and lipids storage in pre-winter period are shown. During this period, starch is observed in medullary rays, perimedullary region and pith (centripetal tendency), while primary cortex, phloem and cambium are rich in lipids (centrifugal tendency).

1 Introduction

On the Earth, according to the data of domestic and foreign taxonomists, wild representatives of *Rosa* L. genus number from 350 up to 500, those growing under conditions of both severe high latitudes and mild climates of tropical and subtropical regions. In XIX century, by inter-specific and inter-sectional hybridization the cultivars were created to become a basement for modern groups of garden roses, which are today as many as about 30 by different gardening classifications. Cultivar pool of the world comprises about 30,000 items [1].

Groups of garden roses with high ornamental qualities such as hybrid teas and floribunda are represented by greatest number of cultivars, of which many provide prospects for cultivating even under severe continental climate conditions of West Siberian forest-steppe. Cultivars of those groups bloom on new growth; even in case of complete withering over-ground parts away during wintering, their annual growth is provided by dormant buds burst in the under-ground part of the plant, which survives winter successfully due to deepen planting elaborated by the gardeners of Tomsk.

Impossibility of saving through wintering a significant amount of shoots is the main limitation for using in Siberian landscape gardening most cultivars belonging to ground-covering or climbing and rambling garden groups, as their flowering occurs at the middle parts of biennial shoots. Histochemical analysis of seasonal dynamics of starch, sugars, proteins and lipids in tissues is one of techniques to diagnose winter hardiness in roses [2].

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Our study targets to assess the level of readiness for wintering in ground-covering roses by dynamics of starch and lipids content in tissues of annual shoots.

2 Materials and Methods

Most of observations and experiments involving ground-covering ornamental plants of various biomorphs have been conducted at the model ground of urban medium on base of Novosibirsk State Agrarian University since 2015. Histochemical techniques were performed in the Laboratory of Introduction of Ornamental Plants of “Central Siberian botanical garden SB RAS”. Samples from USU № 440534 (Collection of alive plants growing indoors and outdoors) were used for experiments. Collaborative investigations of ontomorphogenesis in ground-covering plans are being completed according to the project “Revealing the ways of plants' adaptation to contrast dwelling conditions at the levels of populations and organisms”, State number AAAA-A17-117012610053-9.

Winter hardiness of species and cultivars was assessed by seasonal dynamics of starch and lipids content in tissues of annual shoots [3]. The following compositions of reagents were taken: 1) for starch storage and hydrolysis analyzing – 2 g of potassium iodide, 0.2 g of crystalline iodine and 100 ml of distilled water; 2) to determine lipids – 0.01 g of Sudan III dye and 20 ml of 96 % ethanol, for further microscopy – 10 ml of glycerol.

Scales for quantitative evaluation of storage substances (Table 1) for roses were elaborated by M.V. Besschetnova [4], the entire content being determined in seasonal dynamics by total score for all the tissues.

Table 1. Evaluation of lipids and starch content in shoot tissues for species and cultivars of roses

Starch grains presence in tissues	Tissue staining for different content of lipids	Score
Occur in single cells	Pale-pink, hardly distinctive	1
Occur in less than 50 % of cells	Light-pink, well distinctive	2
Occur in more than 50 % of cells	Pink, of medium intensity	3
Absent in single cells	Bright-pink	4
Present in all cells	Light-red	5

Main objects for the study were the following:

Rosa maximowiczhiana Regel (a selected form, received from Botanical Garden-Institute FEB RAS and plant collected in theit natural habitat at the south of Primorsky Krai). In wild, it is a deciduous shrub with climbing, rambling or creeping stems up to 5 m long. 5-petalled flowers are white. Once-blooming.

Cultivar 'Excelsa' belonging to the group of hybrids of Rose wichuraiana (Welch, USA, 1908). Shoots are 70-95 cm long. Light-green glossy leaves are 8-9 cm long. Rounded buds open into cupped tightly petalled (60 or more petals) flowers of deep pink color, 3–3.5 cm in diameter, arranged in dense inflorescences with mild fragrance. Once-blooming.

Cultivar 'Fuchsia Meidiland' from the group Shrubs (Meilland, France, 1994). Shoots are 65–85 cm long. Glossy leaves are 8.5–10 cm long. Buds have flattened tops. Cupped-to-flat fuchsia-colored flowers are 5.5–6.5 cm in diameter and compose loose inflorescences. Fragrant is mild. Remontant type of blooming.

3 Results

The efficiency of this technique has earlier been shown by us for species and cultivars of roses relating to different morphotypes [5]. Dynamics of lipids and starch content was being analyzed through the period from may to November. However, that was their localization in rose shoot tissues just in the pre-winter period (late November – early December) to be of most interest.

Comparative analysis of ground-covering roses at tissue and organism levels yielded the results below.

The highest winter hardiness was exhibited in *R. maximowiczhiana* samples. Plagiotropic creeping shoots successfully wintered under the natural snow cover. Orthotropic shoots developing throughout the vegetative period were expelled. Though blooming of the wintered shoots was neither abundant, nor continuous. Seasonal dynamics of lipids and starch content and their localization within tissues in pre-winter period are presented in Fig. 1 and Tables 2, 3.

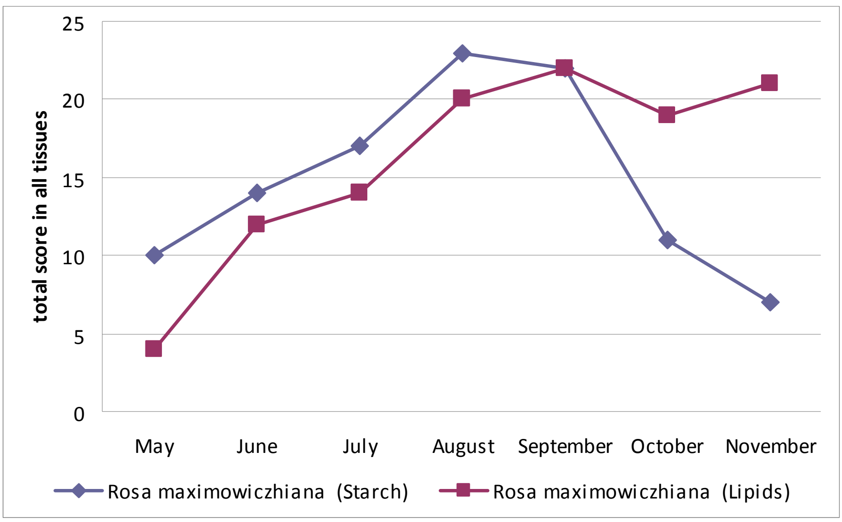

Fig. 1. Seasonal dynamics of lipids and starch content in shoots of winter-hardy ground-covering roses.

Table 2. Starch content in annual rose shoots at their pre-winter stage

Species, cultivar	Primary cortex	Phloem	Cambium	Parenchima of xylem	Medullary rays		Perimedullay region	Pith	Total score
					Uniseriate	Multiseriate			
R. maximowiczhiana	0	0	0	0-1	1	2	1	0-1	4-6
Fuchsia Meidiland	0-1	0	0	1	2	2	1-2	1	8-10
Excelsa	1-2	0	0	1-2	2	2-3	1-2	0-1	11-13

In Tables 2, 3, total score is not calculated automatically for all tissues, but sh varying yearly (that is, there can be 1 point in parenchyma of xylem, or 1 point in uniser medullary rays, but not in both tissues during the same season).

Table 3. Content of lipids in annual rose shoots at their pre-winter stage

Species, cultivar	Primary cortex	Phloem	Cambium	Parenchima of xylem	Medullary rays		Perimedullay region	Pith	Total score
					Uniseriate	Multiseriate			
R. maximowiczhiana	4-5	3-4	2	3	1-3	1	1-3	1-3	20-2
Fuchsia Meidiland	3	2-3	2	2	1	1-2	1	1	13-1
Excelsa	2	1-2	1-2	1-2	1	1	1	0-1	8-10

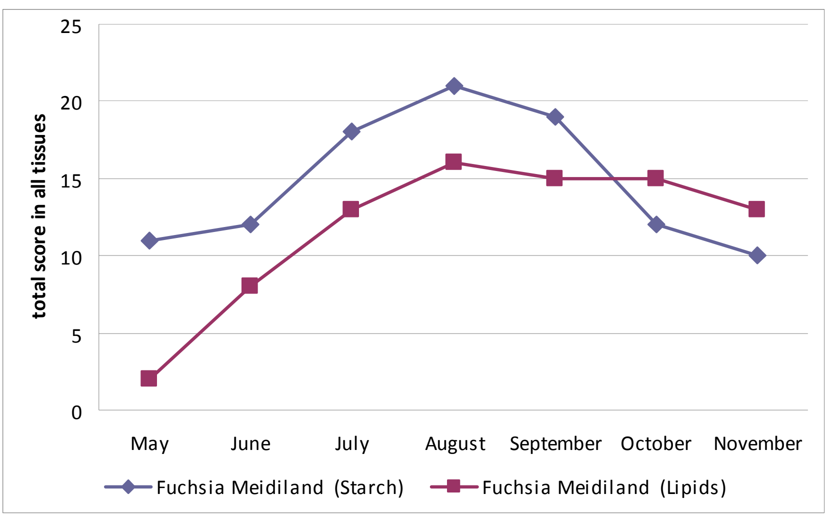

Fig. 2. Seasonal dynamics of lipids and starch content in shoots of ground-covering rose with medium winter hardiness.

Seasonal dynamics of storage substances content reveals that in medium wi hard species *R. maximowiczhiana* outumn starch peak to occurs during the phase of aut foliage coloring (in local winter hard species *R.acicularis* Lindl. and *R. majalis* Herrm maximum is linked to the beginning of abscission). In cultivars with medium and hardiness starck storage maximum is observed at the end of blooming. Actually, varieties under the study do not differ in lipids and starch content in pre-winter perio each other as much as to *R. maximowiczhiana.*

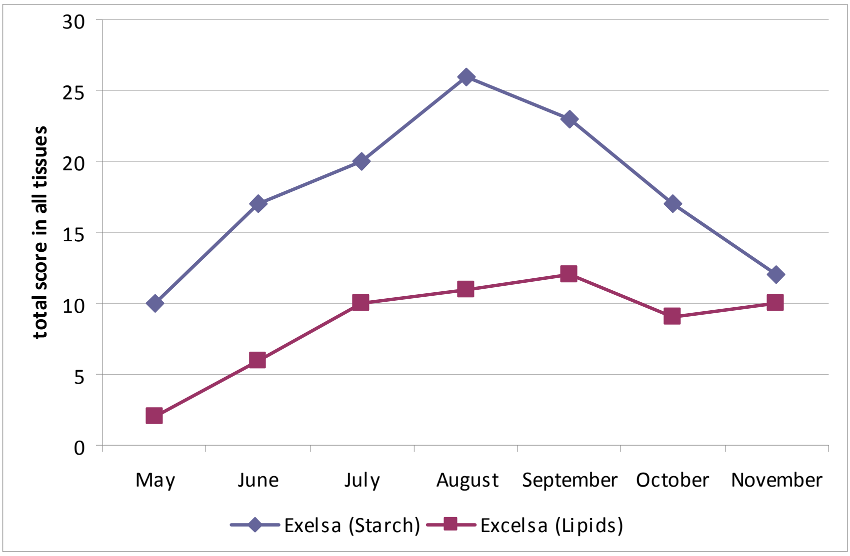

Fig. 3. Seasonal dynamics of lipids and starch content in shoots of ground-covering roses with low winter hardiness

4 Discussion

Nowadays, modern state of investigations in plant hardiness to both high and low temperatures implies application of advanced precision equipment, artificial environment chambers (biotrons); for many studies, model objects, usually herbaceous plants, are used [6–9]. That is of essential importance for profound understanding of acclimatization training mechanisms and cold stress withstanding, as classical histochemical analysis appears insufficient for the purpose. Otherwise, the decided advantage histochemical technique provides is that it enables performing express-assessment of shoot readiness to wintering by quantitative characteristics, in particular starch and lipids localization in certain tissues. The technique does not require expensive reagents, or advanced instrumentation, being widely accessible not only to plant physiologists, but to the ones interested in searching for most resistant gene pools and further breeding and selection.

Seasonal starch transportation within tissues of ground-covering species and cultivars of roses has centripetal pattern; by the end of the vegetative period starch occurs in medullary rays, perimedullary region and pith. On the contrary, lipids are registered in November in primary cortex, phloem and cambium, revealing centrifugal transportation pattern. Shoots of ground-covering roses do not have enough time to complete vegetation, that negatively affecting their winter hardiness.

By and large the group of garden roses under review is not considered as promising for growing under conditions of West Siberian forest-steppe. To expand the possibilities of their cultivation, careful selection of individual cultivars is demanded. An original solution seems to be a combination of two types of ground-covering cultivars – blooming ones and those developing annually the vegetative sphere only.

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