

UDC 582.661.56:577.19:58

**STEM AND LEAF SUCCULENTS WITH CONTRASTING FROST-RESISTANCE  
LEVEL: ACCUMULATION PECULIARITIES OF SOME BIOLOGICALLY ACTIVE  
SUBSTANCES**

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**Introduction**

Plant cultivars capable to keep their ornamental properties during the whole year are quite important aesthetic element of parks and recreational zones of the Crimea and other southern regions. Group of succulents is rather perspective in this point. One of the reasons which makes difficult its wide usage in landscape design is a lack of information about resistance degree to unfavorable environmental conditions. Limited species variety of these ornamentally valuable plants is inconstant way of researching their frost- and winter-resistance, which took place before. Literary sources present data of visual observations [1, 2, 6]. During some years frost-resistance of some specimens from families Crassulaceae and Cactaceae was investigated. As a result of these study cultivars of stem and leaf succulents with a high level of frost-resistance were revealed. Efficiency of introduction work needs not only borders of low-temperature resistance but a number of physiological and biochemical characteristics, which determine development of adaptive syndrome under influence of unfavorable wintering conditions. Such information makes possible to determine a number of characteristics for indirect and objective diagnoses of resistance to unfavorable wintering conditions.

Therefore study objective was to reveal storage peculiarities of some biologically active substances in tissues of stem and leaf succulents with different degree of frost-resistance.

**Objects and research methods**

The following cultivars were studied to investigate storage peculiarities of biologically active substances and their function in development of cryostress for stem and leaf succulents (table 1).

**Frost-resistance of stem (*Opuntia* Mill., *Cylindropuntia* (Eng.) Knuth. Emeng. Backbg.) and leaf (*Sedum* L.) succulents, its characteristic**

Table 1

<i>Sedum</i> genus	<i>Opuntia</i> genus	<i>Cylindropuntia</i> genus
Frost-resistant cultivars		
<i>S. reflexum</i> L. Low critical temperature -16 <sup>0</sup> C... -18 <sup>0</sup> C	<i>O. engelmannii</i> Eng. Low critical temperature -20 <sup>0</sup> C... -22 <sup>0</sup> C	<i>C. leptocaulis</i> (Haw.) Knuth. Low critical temperature – 18 <sup>0</sup> C ... -23 <sup>0</sup> C
<i>S. album</i> L., Genuina Low critical temperature -18 ... -24 <sup>0</sup> C	<i>O. lindheimeri</i> SD. Low critical temperature -18 <sup>0</sup> C... -20 <sup>0</sup> C	<i>C. tunicata</i> (Lehm.) Pfeiff. Low critical temperature -18 <sup>0</sup> C... -20 <sup>0</sup> C
<i>S. acre</i> L. Low critical temperature -18 <sup>0</sup> C... - 20 <sup>0</sup> C		
Mediofrost-resistant cultivars		

<i>S. luteoviride</i> Low critical temperature -10 <sup>0</sup> C...-12 <sup>0</sup> C	<i>O. robusta</i> Wendl. Low critical temperature -15 <sup>0</sup> C...-16 <sup>0</sup> C	<i>C. imbricata</i> (Haw.) Knuth. Low critical temperature -14 <sup>0</sup> C...-18 <sup>0</sup> C
<i>S. palidum</i> L. Low critical temperature -12 <sup>0</sup> C...-14 <sup>0</sup> C	<i>O. leucotricha</i> Eng. Low critical temperature -14 <sup>0</sup> C...-17 <sup>0</sup> C	
<i>S. rubrotinctum</i> R.T. Glausen. Low critical temperature -10 <sup>0</sup> C...-12 <sup>0</sup> C	<i>O. microdasis</i> (Lehm.) Pfeiff. Low critical temperature -12 <sup>0</sup> C ...-15 <sup>0</sup> C	

Methods of artificial shoot freezing in freezing chamber were applied to find out degree of frost-resistance. Gradient of temperature decreasing and increasing was 2°C per hour. Assessment was carried out on 7<sup>th</sup>, 10<sup>th</sup> and 13<sup>th</sup> days [3].

Ascorbic acid concentration in tissue was determined with the help of 0,001 n 2,6 dichlorophenolindophenol solution [5]. Content of phenol compounds was found out photocolimerically with Folin-Chicolteo reagent [5]. Correlation of growth-inhibiting and growth-stimulating substances were determined according to intensity of *Lepidium sativum* seeds sprouting on aqueous-alcoholic extracts made of tissues of study cultivars.

### Results and discussion

As a study results of storage dynamics of ascorbic acid in water-saving tissues of stem and leaf succulents, which belong to *Sedum*, *Opuntia* and *Cylindropuntia* genera, a number of peculiarities was revealed in connection with their cryotemperature resistance (table 2). In particular, concentration of ascorbic acid in tissues of both stem and leaf succulents has two maximum points; the first maximum is connected with blooming period (*Sedum* species – 3<sup>rd</sup> decade of May, 1<sup>st</sup> decade of June; *Opuntia* and *Cylindropuntia* – 1-2 decades of June), the second – with beginning of physiological rest. Frost-resistant cultivars (*S. reflexum*, *O. lindheimtrii* *C. tunicata*) are characterized by quite short vegetative period and as a result – early terms of physiological rest – autumn maximum of ascorbic acid content happens in the 2-3 decades of September.

Table 2

#### Variations of ascorbic acid content in water-saving tissues of stem and leaf succulents characterized by different degree of frost-resistant in a circannian cycle

Cultivars	Ascorbic acid mg % per green weight									
	March	April	May	June	July	August	September	October	November	December
<i>O. engelmannii</i>	21,5± 2,1	26,± 2,6	30,1± 2,6	33,8± 1,9	35,4± 2,0	35,5± 3,0	39,4± 2,3	42,5± 2,6	54,2± 3,0	46,1± 2,7
<i>O. lindheimtrii</i>	23,1± 3,0	24,02± 2,1	22,5± 1,9	29,7± 2,0	35,0± 2,1	25,12± 2,1	25,0± 2,1	34,6± 2,3	43,1± 2,3	38,8± 3,1
<i>O. robusta</i>	27,1± 2,5	25,3± 2,0	24,8± 1,7	27,4± 2,0	45,1± 3,4	37,5± 2,3	35,6± 2,7	34,85 ±3,7	41,7± 3,1	29,31± 2,4
<i>C. tunicata</i>	18,1± 1,4	16,2± 1,3	17,4± 1,1	16,4± 1,5	20,8± 2,5	21,50± 2,1	23,1± 2,3	30,28 ±2,72	31,6± 1,7	27,36± 2,0
<i>C. imbricata</i>	13,0± 1,3	13,5± 1,4	14,0± 2,2	13,8± 1,1	19,2± 1,3	20,6± 1,8	19,4± 1,1	17,2± 1,9	22,1± 2,2	16,6± 1,7
<i>S. reflexum</i>	36,8± 2,3	38,4± 2,5	65,1± 2,6	68,6± 3,3	54,2± 2,8	57,0± 2,6	58,3± 2,6	73,5± 3,7	72,3± 2,9	67,2± 2,4
<i>S. palidum</i>	39,9± 3,5	40,8± 3,0	57,8± 3,4	72,1± 3,2	54,8± 4,9	56,6± 3,9	55,5± 2,9	66,4± 3,8	56,3± 3,4	48,2± 3,3

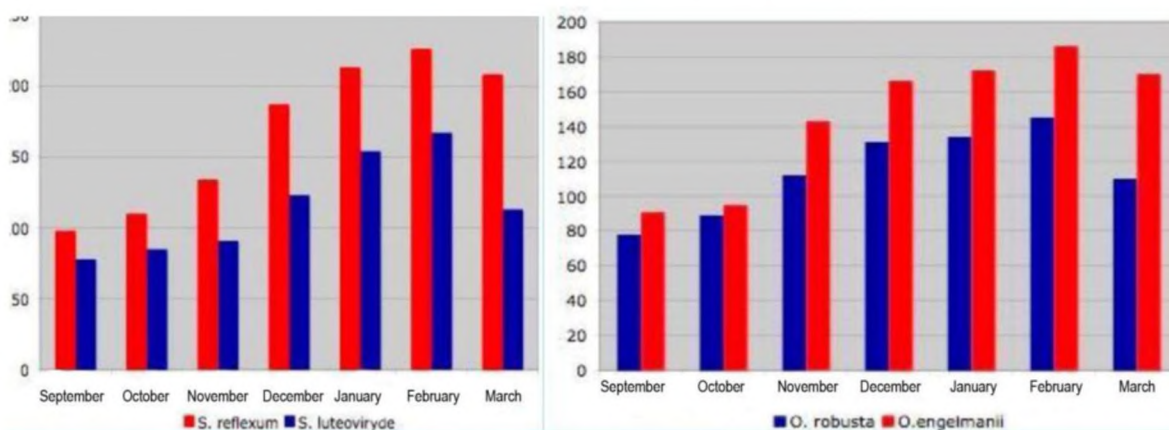
For cultivars with quite low resistance to negative temperatures and continuous vegetative periods this phenomena occurred later – the end of October – beginning of November.

It was determined that stem (*O. engelmannii*, *C. molesta*) and leaf (*S. reflexum*, *S. acre*) cultivars of succulents with high level of frost-resistance are characterized by high content of ascorbic acid in tissues during autumn-winter periods; on average 15-20% more than cultivars with low frost-resistance.

Analyzing concentration of phenol compounds in water-saving tissue of stem and leaf succulents with different level of frost-resistance it was found out, leaf succulents contain more of these substances than stem cultivars. *Sedum* species mainly contains quercetin and its derivatives, while for *Opuntia* and *Cylindropuntia* species principal substances are xanthenes.

Two maximum of phenol compounds storage are registered for both cases: May-June and November-December. Probably, activation of phenol compounds synthesis is connected not only with quantitative but with qualitative factors of these processes. As it's known a number of phenol substances contained in plants are capable to function as growth-controlling factor (depending upon structure and concentration - either inhibiting or stimulating functions) we suppose their storage in spring period is caused by beginning of growth processes, while in November-December – by realization of adaptive mechanisms to negative temperatures effect. Experiments results (sprouting of *Lepidium sativum* seed on aqueous-alcoholic extracts made of tissues of succulents, which contain phenol compounds) can prove this conclusion. It was found out that if air temperature regime has steady tendency of decreasing passing point of +5°C (the 1<sup>st</sup> decade of November) tissue extracts of frost-resistant cultivars *Sedum* and *Opuntia* increase concentration of growth-inhibiting substances while cultivars with low frost-resistance this phenomena takes place after first frosts (the 2<sup>nd</sup> decade of November).

It turned out that frost-resistant cultivars, either stem (*O. engelmannii*) or leaf (*S. reflexum*) succulents, have more intensive synthesis of phenol compounds in comparison with not so frost-resistant cultivars such as *O. robusta* and *S. palidum* (Fig.1).



**Fig.1 Concentration of phenol compounds in tissues of cultivars of *Opuntia* u *Sedum* genera characterized by different degree of frost-resistance**

These findings allowed to conclude frost-resistance of stem (cultivars of *Opuntia*, *Cylindropuntia* genera) and leaf (cultivars of *Sedum* genus) succulents is caused by peculiarities of their secondary metabolism: dynamics of ascorbic acid and phenol compounds

storage in water saving tissues. Tissues of plant cultivars with relatively high cryotemperature resistance contain 20-25% more during cold period than cultivars with low resistance to negative temperature do.

Analysing long-term observation of dynamics the ascorbic acid storage in study cultivars it was concluded that its concentration depends upon weather conditions of a certain year. So, warm winter 2012-2013 with a high humidity but without hardening temperature parameters caused decreasing of frost-resistance degree of stem and leaf succulents on average this difference made 5...8°C, that's why ascorbic acid storage in tissues of wintering organs decreased its intensity (Fig.2).

Nevertheless, thanks to two contrasting by cryotemperature-resistance study cases of *Opuntia* genus it was revealed that tendency to more intensive storage of ascorbic acid in tissues of frost-resistant cultivars keeps its parameters.

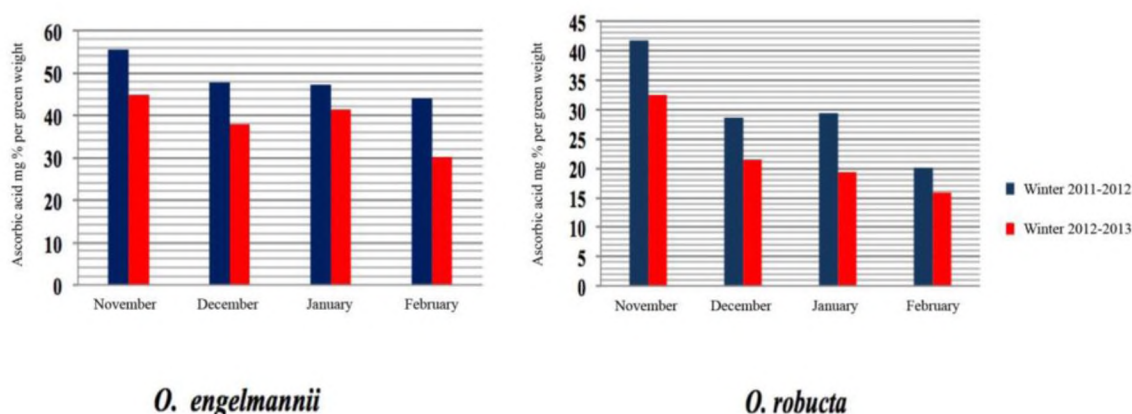


Fig.2 Ascorbic acid concentration in tissue of cultivars of *Opuntia* genus during winter periods of 2011-2012 and 2012-2013

Similar pattern takes place in comparison of phenol compounds in tissues of study cultivars during winter periods of 2011-2012 and 2012-2013. Probably there is cryoprotecting function of this substances with their antioxidant properties and possible participation in control of lipids peroxidation.

### Conclusions

1. In terms of this research it was found out that ascorbic acid concentration in tissue of succulents has two maximum points, the first one is connected with blooming period (*Sedum* species – the 3<sup>rd</sup> decade of May – the 1<sup>st</sup> decade of June; species of *Opuntia* and *Cylindropuntia* – the 1-2<sup>nd</sup> decades of June), the second maximum point is explained by beginning of physiological rest (depending upon cultivar classification – October-November).

2. Frost-resistant cultivars of *Sedum*, *Opuntia* and *Cylindropuntia* genera are characterized by increasing of phenol compounds concentration in the beginning of cold period. Maximum content of phenols of stem and leaf succulents was registered in the coldest month on South Coast of the Crimea – February.

3. It was revealed that endogenic growth-regulators and frost-resistance level are connected. If air temperature regime has steady tendency to decreasing passing the point of 10°C (the 2<sup>nd</sup> and 3<sup>rd</sup> decades of November), tissues of frost-resistant cultivars of *Sedum* and *Opuntia* increase concentration of growth-inhibiting substances while cultivars with low frost-resistance have this phenomena after daily average temperatures go down passing the point of +5°C (the 2<sup>nd</sup> decade of December).

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*The article was received at editors 12.11.2014*

**Gubanova T.B. Stem and leaf succulents with contrasting frost-resistance level: accumulation peculiarities of some biologically active substances** // Bull. of the State Nikit. Botan. Gard. – 2015. – № 115. – P. 54-58.

The article presents dynamics results of ascorbic acid and phenol compounds storage in tissue of cultivars of *Sedum*, *Opuntia* and *Cylindropuntia* genera with contrasting frost-resistance level. It was found out that cultivars with a high resistance to low temperature have synthesis of phenol compounds and ascorbic acid activated as soon as cold season begins. Perhaps these substances participate in protective mechanisms under conditions of low-temperature stress.

**Key words:** *frost-resistance; succulents; ascorbic acid; phenol compounds.*

### MYCOLOGY

UDC 502.73:582.28(477.75)

### NEW FUNGAL SPECIES INHABITING ON *DAPHNE TAURICA* KOTOV (THYMELAEACEAE) IN THE CRIMEA

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### Introduction

*Daphne taurica* Kotov is an endemic plant of the Crimea. It is a deciduous shrub 40-80 sm (120 sm), stems in the bottom part can reach 28 mm across diameter. Bark on stems and lower branches is dark-brown, last-year`s branches have dark-purple color, shoots of the current year are greenish. Leaf length makes 4-48 mm, width – 2-10 mm. Leaves are sessile, bare, leathery, quite hard, oblong - inversely egg-shaped, edges are folded, bottom is wedge-shaped short (large leaves) oblong without visible footstalk. Flowers are aromatic, yellow-white or cream-colored by length of 1-3 sm, densely growing and shape heads (3-9 flowers) on the ends of short fruiting woods with thick foliage. Bloom period is in May-June. Fruits are dark red ripened drupes, young fruits have red color. Fruiting takes place in August [2, 4, 5].