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EVALUATION OF THE DROUGHT RESISTANCE OF GARDEN ROSES BASED ON THE PROLINE CONTENT, WATER RETENTION CAPABILITY AND STATE OF CELL MEMBRANES UNDER THE INFLUENCE OF HYDROTHERMAL STRESS

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To identify the drought resistance of seven garden roses genotypes during the period of maximum probability of drought on the Southern Coast of the Crimea, the water regime parameters, proline content and permeability of cell membranes were studied under conditions of controlled dehydration. It was revealed that different conditions of hydrothermal stress have different effects on the studied genotypes of garden roses. In relatively soft wilting conditions, after stress relief, the proline concentration decreased, reaching almost control values and permeability of cell membranes of the leaves of most of the studied objects, except *Rosa gallica* and *Rosa chinensis var. minima*, was not impaired. Under conditions of simulated dry weather, after restoration of water supply, the proline concentration and permeability of leaf cell membranes of most genotypes were increased, which indicates damage or partial death of leaf tissues. The destruction of cell membranes occurred in the leaves of the *Rosa gallica* species, which led to the complete death of tissues. Based on the resistance of cell membranes to destruction and cell protoplasm to dehydration, it was found that 'Borisfen' cv. and *Rosa hugonis* differ in maximum drought resistance.

Key words: *drought resistance; controlled wilting; water regime parameters; proline content; permeability of cell membranes; release of electrolytes; roses*

Introduction

On the Southern Coast of the Crimea (SCC), which belongs to the subhumid zone, dry summer seasons are accompanied by an increase in air temperature to 37°C, which negatively affects the vital function and decorative properties of plants [16]. The problem of the influence of drought on the state of plants has become particularly relevant in connection with the aridization of territories in a number of southern regions, due to global climate change. In recent decades, almost every year there has been a simultaneous manifestation of soil and air droughts in the SCC. Since the cultivation of garden crops is limited by a shortage of irrigation water in the summer, special attention is paid to the identification of drought-resistant genotypes. The garden rose is of interest for research as one of the most decorative crops, which allows solving various tasks in the landscape design of the SCC [15].

By now, data have been obtained that among the numerous physiological and biochemical mechanisms that ensure the adaptive properties of plants, a significant role belongs to the biomembrane system [6, 14]. High cell activity and stability of cell membranes, an indicator of which is the low permeability of protoplasm to electrolytes, ensure high heat and drought resistance of plants under water stress [3, 8]. The stability of a cell membrane is a physiological indicator widely used to assess drought resistance [11, 17]. Since the output of electrolytes depends on the degree of damage to cellular structures, assessing the stability of cell membranes in leaf tissues based on exosmosis measurements after stress has become a widespread method for assessing cell viability [18, 19].

Proline is one of the most effective osmoprotectants and signaling molecules due to its cyclic structure and a secondary amino group distinct from the proteinogenic amino acids. In addition, proline has an essential role in primary metabolism in the forms of free amino acids and as part of proteins [4, 20]. Proline is an important part of the physiological adaptation of many plant species to stress and does not damage cellular structures, accumulating in the cytosol [9]. Proline can protects membranes integrity and improve protein stability by binding to hydrogen bonds [10]. Also, it may protect cells by facilitating activation of enzymes and increasing water uptake potential [4]. Proline is also considered as a potent antioxidative defense molecule, a metal chelator, a ROS scavenger, and an inhibitor of programmed cell dying [1]. Numerous data suggest the positive correlation between the content of proline and level of stress tolerance in the plants [5, 13].

The objective of the work is to specify the level of drought resistance of seven genotypes of garden roses by level of proline content and permeability of cell membranes affected by hydrothermal stress under controlled conditions.

Materials and methods

The objects of the study were the leaves of seven genotypes of garden roses: species *Rosa bracteata* J.C. Wendl., *Rosa chinensis* var. *minima* (Sims) Voss., *Rosa hugonis* Hemsl., *Rosa foetida var. persiana* (Lem.) Pehder, *Rosa gallica* L., as well as complex interspecific hybrids: *Rosa odorata* var. *gigantea* Reder & Wilson × *Rosa multiflora* Thunb. and 'Borisfen' cv. (*Rosa fedtschenkoana* Regtl. × Kordes Sondermeldungis) growing in the collection areas of the Nikitsky Botanical Gardens.

To assess the level of drought resistance of garden roses genotypes, a series of experiments were conducted under controlled conditions of leaf wilt at various combinations of temperature and humidity: variant 1 – air temperature +22°C, relative humidity 50%; variant 2 – air temperature +30°C, relative humidity 70%; variant 3 – air temperature +30°C, relative humidity 30%; variant 4 – air temperature +35°C, relative humidity 25%. The output of electrolytes and proline content in the leaves was determined in a state of complete watering, after 3-5 hours of wilting and after restoration of water supply of leaf tissues. Leaves in a state of complete watering were used as a control sample. The experiments were carried out in Binder MKF 115 climate chamber. The permeability of cell membranes was assessed by the release of electrolytes from leaf carvings into distilled water using a portable Ohaus Starter 300 conductometer. The actual electrolyte yield was calculated as a percentage of the total yield [7]. Proline content was determined according to the modified Chinard method [2]. The water-retaining forces and reparative capabilities of the leaf apparatus were determined using the methodological recommendations of A.L. Lischuk [12].

The experiments were carried out in the 3-fold repetition. The MS Excel 2007 software application was used for statistical processing of the data obtained. The table shows the average values of the definitions and their standard errors.

Results and discussion

Due to the fact that the realization of potential resistance to moisture loss significantly depends on the weather conditions of the summer season, a series of experiments were conducted on the controlled wilting of garden rose leaves at various combinations of temperature and humidity. As hydrothermal stress in natural conditions increased, the conditions of controlled wilting became tougher every month.

At the end of May, the water content in the leaves of the studied roses varied between 62.3-69.5% of the wet weight, the level of real water deficiency was 9.5-12.2% (table 1). The genotypes *Rosa chinensis var. minima* and *Rosa odorata* var. *gigantea* × *Rosa multiflora* differed by relatively high indicators of hydration of leaf tissues, along with minimal water deficiency. In the fully hydrated leaves of the studied genotypes, the proline content ranged from 87.7 to 214.6 µg/g (fig. 1). When leaves were completely watered, most genotypes of the rose had an electrolyte yield of 6.0-9.9%, with the exception of the species *Rosa gallica* (12.9%) (table 1). Thus, in the leaves of *Rosa gallica* species, disfunctions in the state of cell membranes manifested themselves already with complete watering of the leaves. In the process of wilting under mild conditions (t 22°C and Rh 50%), the increased water-retaining forces of the leaves were demonstrated by the genotypes *Rosa odorata* var. *gigantea* × *Rosa multiflora*, *Rosa hugonis* and 'Borisfen' cv., which lost 15.4-20.0% moisture in 7 hours of wilting. After dehydration in the leaves of the above species, the proline content increased by 185-247% and the output of electrolytes did not exceed 10%, which indicates a stable state of cell membranes.

Table 1

Parameters of the water regime and changes in the permeability of cell membranes of garden rose leaves under various conditions of hydrothermal stress

Genotype	Water content of the leaves, %	Water deficit, %	Experiment variant	Electrolyte output, %		
				Control (complete watering)	Wilting	Restoration
Rosa bracteata J.C. Wendl.	63.24±1.6	10.95±0.9	1	8.9±0.3	6.8±0.2	5.9±0.2
	62.31±1.2	11.59±1.1	2	8.9±0.3	16.5±0.6	8.2±0.3
	51.20±1.0	17.54±1.5	3	6.2±0.2	22.0±0.7	11.3±0.4
	51.82±1.1	13.31±1.3	4	13.3±0.4	18.3±0.6	17.9±0.6
Rosa chinensis var. *minima* (Sims) Voss.	69.54±2.1	10.00±0.8	1	9.8±0.3	10.9±0.4	9.0±0.3
	62.81±1.9	17.65±1.6	2	7.9±0.3	24.3±0.8	10.7±0.4
	59.75±1.3	31.09±2.3	3	16.4±0.6	30.6±1.0	14.8±0.5
	61.15±1.5	10.09±1.1	4	13.2±0.4	18.2±0.6	20.1±0.7
Rosa foetida var. *persiana* (Lem.) Pehder	62.32±1.7	12.20±1.3	1	9.9±0.3	15.3±0.5	7.2±0.2
	60.00±1.2	19.23±1.4	2	11.4±0.4	16.2±0.5	8.3±0.3
	57.66±1.4	30.60±2.0	3	8.6±0.3	17.3±0.6	11.8±0.4
	56.76±1.1	19.23±1.6	4	10.3±0.3	21.8±0.7	11.4±0.4
Rosa gallica L.	62.44±1.8	11.16±1.2	1	12.9±0.4	30.0±1.0	16.4±0.5
	58.50±1.3	19.54±1.4	2	14.5±0.5	27.2±0.9	14.2±0.5
	56.68±1.5	26.38±1.7	3	20.2±0.7	40.8±1.2	27.9±0.9
	52.63±1.2	29.89±1.9	4	19.6±0.7	39.6±1.3	40.9±1.3
Rosa hugonis Hemsl.	65.69±1.7	10.76±1.3	1	6.0±0.2	9.0±0.3	5.6±0.2
	61.51±1.1	13.42±1.0	2	8.1±0.3	17.2±0.6	6.7±0.2
	55.61±0.7	20.27±1.2	3	8.1±0.3	16.4±0.6	11.2±0.4
	56.61±0.9	18.32±1.5	4	9.5±0.3	22.2±0.8	11.2±0.4
Rosa odorata var. gigantea × *Rosa multiflora*	69.01±1.6	9.50±0.6	1	6.6±0.2	6.3±0.2	6.8±0.2
	67.33±1.8	10.02±0.8	2	8.2±0.3	19.4±0.7	5.9±0.2
	63.97±1.3	19.95±1.3	3	8.9±0.3	19.9±0.7	12.0±0.4
	58.65±1.0	17.69±1.1	4	11.0±0.4	19.1±0.6	16.5±0.6
'Borisfen'	66.35±1.5	11.80±0.7	1	7.4±0.2	9.5±0.3	9.6±0.3
	63.74±1.2	14.28±0.9	2	9.9±0.3	17.5±0.6	8.7±0.3
	61.73±1.1	18.26±1.2	3	10.0±0.3	18.6±0.6	11.0±0.4
	58.09±0.8	22.30±1.4	4	14.8±0.5	21.0±0.7	12.6±0.5

The weakest water-retaining forces were shown by the leaves of *R. gallica* and *R. chinensis* var. *minima* – after three hours, the moisture loss reached 22%. The leaves of *R. foetida* var. *persiana* also showed low water retention, having lost 24% of water after four hours of wilting. In a state of wilting, the roses leaves of the above species showed an increased proline content. The increase varied from 95% (*R. chinensis* var. *minima*) to 147% (*R. foetida* var. *persiana*). The electrolyte yield slightly exceeded the control values in the leaves of *R. chinensis* var. *minima* species (10.9%) and increased more significantly in the leaves of *R. foetida* var. *persiana* (15.3%), which demonstrates impaired permeability of cell membranes, causing damage to leaf tissues. In the leaves of *R. gallica*, the exosmosis reached 30.0%, which may indicate significant disfunctions in the permeability of cell membranes, leading to tissue death. After restoration of moisture availability in *R. indica*, *R. hugonis* and 'Borisfen' cv., the level of leaf surface area repair was 100%, 85% and 95%, respectively. The leaves of *R. chinensis* var. *minima* and *R. foetida* var. *persiana* showed weak repair capabilities: no more than 70% and 60% of the leaf surface area were restored, respectively. After rehydration, the concentration of proline in leaves of most genotypes decreased, continuing to increase in *R. gallica* species. At the same time, the electrolyte yield in the leaves of all studied genotypes decreased. The leaves of most of the studied rose genotypes demonstrated a good state of cell membranes – the electrolyte yield was less than 10%. The exception was the leaves of *R. gallica* species, the exosmosis of which was 16.4%, which indicates an increase in the permeability of cell membranes, leading to damage or partial death of tissues.

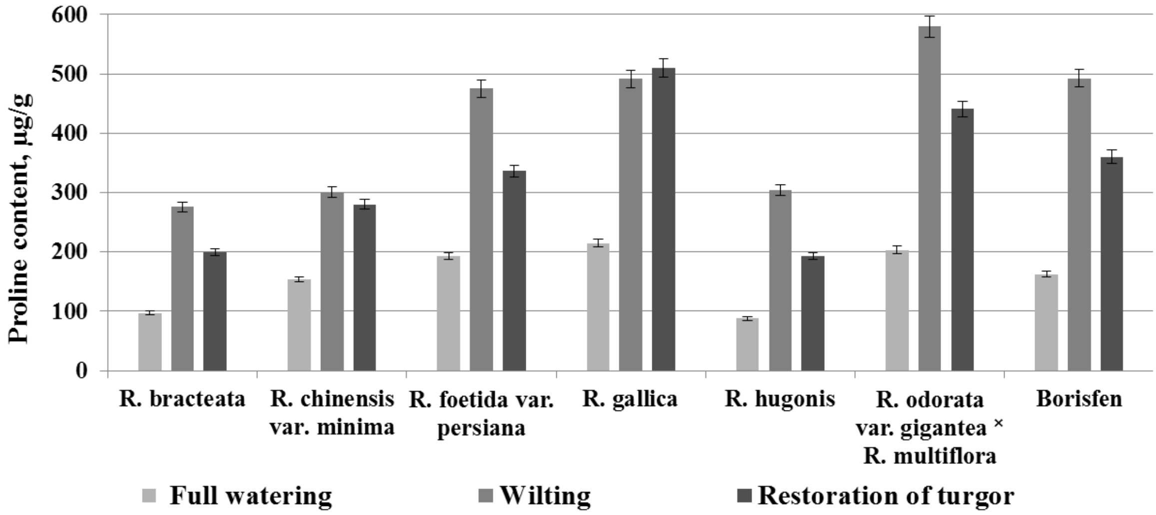

Fig. 1 Proline content in roses leaves under controlled wilting at air temperature +22°C, relative humidity 50%

In June, leaf hydration decreased to 58.5-67.3%, which is 2-7% lower than in May (table 1). Moisture deficiency increased and ranged from 10.0% (in *R. odorata var. gigantea* × *R. multiflora*) to 19.5% (*R. gallica*). In the state of complete watering in the roses leaves, the proline concentration ranged from 94.7 to 219.5 µg/g (fig. 2). The outflow of electrolytes of most genotypes was 7.9-9.9% (table 1). The exception was the species *R. gallica* and *R. foetida* var. *persiana*, whose leaf exosmosis was 14.5% and 11.4%, respectively, which indicates a disfunction of the permeability of cell membranes. In more severe wilting conditions (t 30ºC and Rh 70%), the leaves of the species *R. odorata* var. *gigantea* × *R. multiflora*, *R. hugonis* and 'Borisfen' cv. showed the maximum water retention capacity – after 8 hours, the water loss did not exceed 22%. The leaves of *R. bracteata* and *R. foetida var. persiana* species lost a similar amount of moisture in 6 hours, thus showing a high water retention capacity. The leaves of *R. gallica* showed minimal moisture retention: during the first hour of wilting, they lost about 30% of moisture, which led to partial tissue death. After the leaves withered at a temperature of 30°C and humidity of 70%, the proline content in the leaves of roses increased more intensely: from 160% (*R. chinensis* var. *minima*) to 350% ('Borisfen' cv). In more severe wilting conditions, the electrolyte yield reached 16.2-27.2%. The leaves of *R. foetida* var. *persiana* and *R. bracteata* differed in the minimum permeability of cell membranes, and the maximum permeability was *R. chinensis* var. *minima* and *R. gallica*. After the restoration of the water supply, good repair capabilities were demonstrated by the genotypes *R. odorata* var. *gigantea* × *R. multiflora*, *R. hugonis*, *R. bracteata*, *R. foetida* var. *persiana* and 'Borisfen' cv. – the leaves restored turgor by 85-100%. When restoration of moisture availability, the proline concentration in leaves of most genotypes decreased, reaching almost control values. Only in the leaves of *R. gallica* and *R. chinensis* var. *minima* species did the proline content continue to increase. After the end of the stress effect, the level of electrolyte output in the leaves of most of the studied genotypes decreased below the control values. The exception was the species *R. gallica* and *R. chinensis* var. *minima*, in whose leaves, after the end of stress, exosmosis was 14.2% and 10.7%, respectively, which shows disfunctions of the permeability of cell membranes.

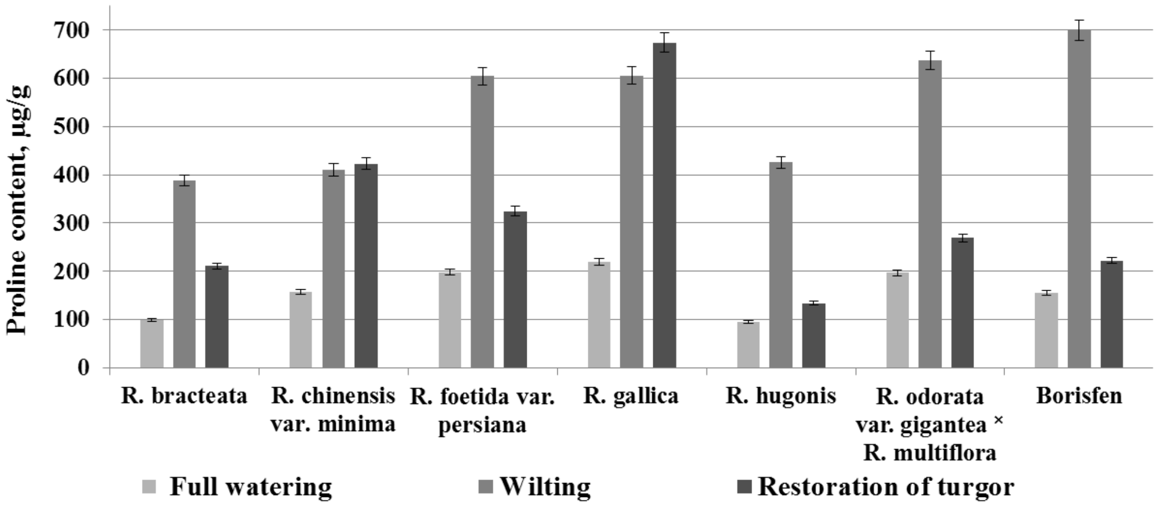

Fig. 2 Proline content in roses leaves under controlled wilting at air temperature +30°C, relative humidity 70%

In July, the water content in rose leaves decreased to 51.2-64.0% (2-11% lower than the previous month), moisture deficiency increased, most significantly in the tissues of the leaves of *R. chinensis var. minima* – by 13%, *R. foetida* var. *persiana* – by 11%, *R. odorata var. gigantea* × *R. multiflora* – by 10% (table 1). In the state of complete leaf watering, the content of proline ranged from 104.4 to 244.6 µg/g (fig. 3).

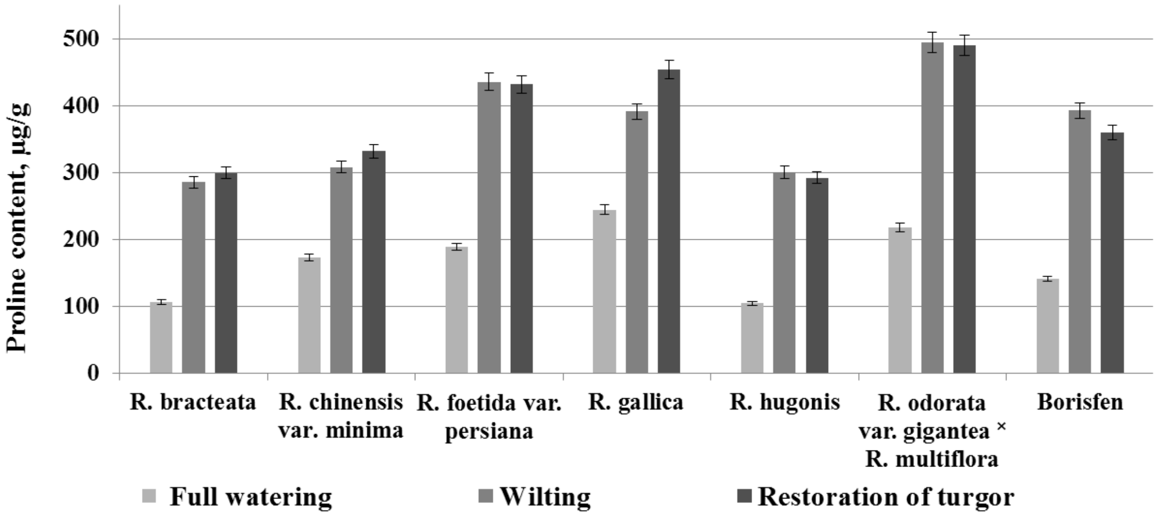

Fig. 3 Proline content in roses leaves under controlled wilting at air temperature +30°C, relative humidity 30%

The electrolyte yield in most genotypes, with the exception of *R. gallica* (20.2%) and *R. chinensis* var. *minima* (16.4%) species, did not exceed 10% (table 1). Under wilting conditions close to the action of dry wind (t 30°C and Rh 30%), the process of moisture release by the leaves was more intense: the studied genotypes, with the exception of 'Borisfen' cv., lost from 24% to 30% of water after 2 hours, and *R. gallica* – 40% within 1 hour. The highest water-retaining forces were demonstrated by 'Borisfen' cv.: the loss of moisture by the tissues of its leaves after 4 hours was only 22%. During leaf wilting at high temperature and low humidity, the concentration of proline in the roses leaves increased less than at high humidity. The increase varied from 60% (*R. gallica*) to 188% (R. *hugonis*). After the leaves withered at a temperature of 30°C and humidity of 30%, the electrolyte yield in most genotypes was 16.4-22.0%, which indicates an increase in the permeability of cell membranes. In the leaves of the species *R. gallica* and *R. chinensis* var. *minima*, the exosmosis reached 40.8 and 30.6%, which is an indication of damage to the membranes of leaf tissues. After the restoration of water supply, the amount of leaf surface area of the studied genotypes that restored normal turgor, with the exception of *R. gallica*, varied from 80% to 100%. After restoration of moisture availability, the proline concentration in leaves decreased only for 'Borisfen' cv., continuing to increase in *R. gallica* and *R. chinensis* var. *minima* species. In the leaves of the other studied genotypes, the proline content remained virtually unchanged. The exosmosis of the leaves of the studied rose genotypes varied in the range from 11.0 to 27.9%, which indicates an increase in membrane permeability and, as a result, tissue damage or partial death.

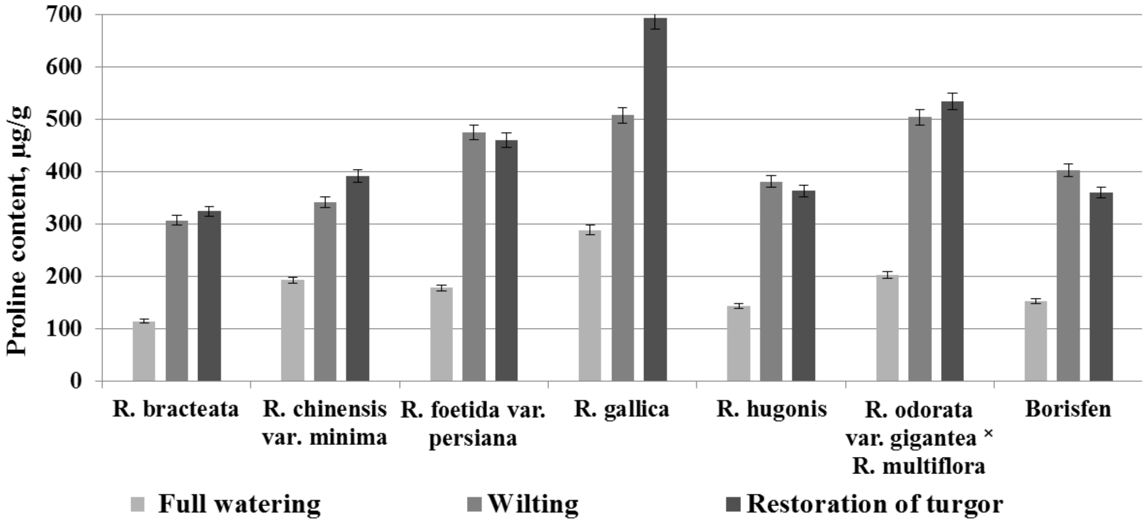

Fig. 4 Proline content in roses leaves under controlled wilting at air temperature +35°C, relative humidity 25%

In August, the hydration of the leaves of most of the studied objects did not undergo significant changes, only *R. gallica*, *R. indica* and 'Borisfen' cv. showed a decrease in water content by 4-5%, and therefore, water deficiency increased in the tissues of *R. gallica* and 'Borisfen' cv. (table 1). When leaves were completely watered, the content of proline ranged from 114.3 to 288.8 µg/g (fig. 4). In the fully hydrated leaves of most of the studied genotypes, the electrolyte yield varied from 10.3 to 14.8%, which shows increased permeability of cell membranes (table 1). In the leaves of *R. hugonis* species, the exosmosis was 9.5%, and in the leaves of *R. gallica* species it reached 19.6%. In conditions of simulated dry weather (t 35°C and Rh 25%), after three hours of wilting, rose leaves gave off from 26% to 29% moisture. The leaves of *R. gallica* species lost 35% of their water in 1 hour. During leaf wilting at higher temperature and lower humidity, the proline content in the leaves increased more intensely: from 76% (*R. gallica*) to 167% (*R. foetida* var. *persiana*). After the leaves withered, the electrolyte yield in most genotypes increased by 5-20% and amounted to 18.2-22.2%, in the species *R. gallica* – 39.6%. After saturation, it was found that only in the leaves of *R. foetida* var. *persiana* species, tissue turgor was almost completely restored (95%), despite significant water loss (27%). The rest of the genotypes have a level of repair, with the exception of *R. gallica*, made up from 75% to 90% of the leaf surface area. After rehydration, the concentration of proline in leaves of most genotypes did not significantly change. The proline concentration in leaves decreased only for 'Borisfen' cv., continuing to increase in *R. gallica* and *R. chinensis* var. *minima* species. After the cessation of stress, the permeability of cell membranes of the leaves of most genotypes remained elevated – the outflow of electrolytes ranged from 11.2 to 20.1%. The exception was the species *R. gallica*, in whose leaves the electrolyte yield reached 40.9%, which reveals a sharp increase in membrane permeability, leading to complete tissue death.

Conclusion

The assessment of water regime parameters, changes in proline concentration and the state of cell membranes of the leaves of some genotypes of garden roses under conditions of control wilting is given. Studies of these parameters in the leaves of garden roses under the controlled conditions have shown that different conditions of hydrothermal stress cause different reactions in the studied genotypes.

When stress was revealed after wilting in relatively soft conditions, the proline concentration and the permeability of cell membranes of the leaves of most of the studied genotypes was restored, returning to a stable state. Increases in membrane permeability leading to damage or partial death of leaf tissues were detected only in the species *R. gallica* and *R. chinensis* var. *minima*. In addition, in the leaves of only these species, the proline content continued to increase after the stress ceased. Minimal damage to cell membranes, along with high water retention abilities, were observed in the leaf tissues of *R. hugonis*, *R. odorata* var. *gigantea* × *R. multiflora*, and 'Borisfen' cv. Additionally, under drought conditions, proline was substantially more accumulated in the leaves of the *R. hugonis* and 'Borisfen' cv. Therefore, these genotypes showed an osmotic adaptation mechanism that enables them to withstand short-term drought stress.

In the conditions of simulated dry weather, after stress relief, the proline concentration and cell membranes permeability of most genotypes leaves were increased, which indicates damage or partial death of leaf tissues. In the leaves of *R. gallica* species, a sharp increase in membrane permeability indicates their destruction and, accordingly, complete tissue death. In conditions close to dry weather, the membranes of leaf cells of *R. hugonis*, *R. foetida* var. *persiana* and 'Borisfen' cv. were minimally damaged. After restoration of moisture availability, the proline concentration in leaves decreased only for 'Borisfen' cv.

It was revealed that 'Borisfen' cv. and *R. hugonis* species are the most drought-resistant, due to the resistance of cell membranes to destruction and cell protoplasm – to dehydration.

For rose genotypes, the water-retaining forces, proline concentration and cell membrane integrity were effective criteria for detecting drought tolerance strategies, while the water content and water deficit did not show any significant relation with drought tolerance.

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Гребенникова О., Пилькевич Р., Плугатарь С. Оценка засухоустойчивости садовых роз на основе содержания пролина, влагоудерживающей способности и состояния клеточных мембран под влиянием гидротермального стресса // Бюллетень Государственного ботанического сада – 2025. – № 157. – С. 64-71.

Для выявления засухоустойчивости семи генотипов садовых роз в период максимальной вероятности засухи на Южном берегу Крыма были изучены параметры водного режима, содержание пролина и проницаемость клеточных мембран в условиях контролируемого обезвоживания. Было выявлено, что различные условия гидротермального стресса по-разному влияют на изучаемые генотипы садовых роз. В условиях относительно мягкого увядания, после снятия стресса, концентрация пролина снижалась, достигая почти контрольных значений, а проницаемость клеточных мембран листьев большинства изучаемых объектов, за исключением *Rosa gallica* и *Rosa chinensis* var. *minima*, не нарушалась. В условиях имитируемой сухой погоды, после восстановления полива, концентрация пролина и проницаемость клеточных мембран листьев большинства генотипов увеличились, что указывает на повреждение или частичную гибель тканей листьев. В листьях вида R*osa gallica* произошло разрушение клеточных мембран, что привело к полному отмиранию тканей. Основываясь на устойчивости клеточных мембран к разрушению и клеточной протоплазмы к обезвоживанию, было установлено, что ‘Borisfen’ cv. и *Rosa hugonis* отличаются максимальной засухоустойчивостью.

Ключевые слова: *засухоустойчивость; контролируемое увядание; параметры водного режима; содержание пролина; проницаемость клеточных мембран; выделение электролитов; розы*