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Physiological and biochemical features of some cultivars in essential oil rose (*Rosa* × *damascena* Mill.) growing *in situ* and *in vitro*

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Abstract: The results of physiological and biochemical features studies in some essential oil rose cultivars from Nikita Botanical Gardens collection under cultivation *in situ* and *in vitro* are presented. Studies of the intact plants were carried out in their growth dynamics. For the cleaning up of valuable cultivars, explants of studied plants were introduced to *in vitro* culture. Meristems 0.2-0.4 mm long were isolated from vegetative buds and placed to the modified Murashige and Skooge (1962) medium, supplemented with plant growth regulators. The culture was maintained under controlled conditions: temperature 24 ± 1°C, light intensity 25.0-37.5 μmol m^{-2} s^{-1} and 16-hour photoperiod. The samples were tested after 9-12 months of the culture. Our results demonstrated that studied cultivars of essential oil rose had a high degree of adaptation both *in vitro*, and *in situ*. These properties are provided by xeromorphic structure of vegetative organs, high water-holding capacity of leaf tissue due to the bound water fraction. Formation of rose plants` protective response to the hydrothermal stress and further adaptation response were highly depended on various protective compounds (free proline, phenolic compounds, ascorbic acid) accumulation. Thus, in different rose cultivars the level and rate of protective compounds accumulation differed. Effects of hydrothermal stress resulted in catalase activity decrease while superoxide dismutase and polyphenol oxidase activity increased in the rose cultivars Festivalnaya, Lany and Raduga. These changes were associated with the ability of these cultivars to adapt to the quickly changing environmental conditions. Based on the research results we could recommend essential oil rose cultivars Raduga and Festivalnaya for mass cultivation in the regions with semi-arid climate and use in the essential oil industry.
Key words: essential oil rose, hydrothermal stress, water regime, photosynthetic activity, protector substances, oxidoreductase activity.

Introduction

Plants during ontogeny grow in a changeable environment and are constantly exposed to a wide range of abiotic and biotic factors, including relevant fluctuation of temperature and moisture conditions during the growing season in the south of Russia. Essential oil rose (*Rosa* L.) is one of the main essential oil crops. Rose flowers, rose water and rose essential oils are used in confectionery, medicine, perfumery and cosmetic industry[1]. The limiting factor of vegetative propagation of this culture is its high susceptibility with viral pathogens. It was found that in addition to phenotypic changes, viruses cause disturbance in the functional status of various plant species and cultivars. Complex biotech system of virus diagnostics and cleaning up allows to get high-quality plant material [2,3].

However, adaptation to new culture conditions, particularly: transfer of plant material from the aseptic culture conditions (*in vitro*) into the greenhouse (*in vivo*) and field (*in situ*) is complex and it is based on cytological and anatomical plasticity of plant structures, liability and tolerance of biochemical and physiological parameters, the limits of which depend on the genotype[4].

Under the influence of unfavorable environmental factors on plants redox processes are important. One of the earliest responses to the stress is active oxygen forms (AOF) formation. Having high reactive ability AOF disrupt many processes in the cells, as well as its structures[5]. To prevent such cell disturbances exist in antioxidant systems comprising both non-proteinaceous low molecular weight antioxidants (proline, ascorbic acid, flavonoids, etc.) and specific enzymes – antioxidants (superoxide dismutase (SOD), catalase, various oxidases, including polyphenoloxidase (PPO) and etc.). The studies of antioxidant systems functioning is important for understanding ways of adaption in plants to the changed environmental conditions [6, 7].

Thus, the objective of this work was to evaluate physiological and biochemical indexes in plants of some essential oil rose cultivars from the Nikita Botanical Gardens – National Scientific Center (NBG-NSC) collection under various cultivation conditions and to select cultivars with the greatest adaptive capacity.

Materials and Methods

Studies of the intact plants were carried out during the growing season (June - August). For physiological and biochemical studies leaves from the middle part of shoots were collected. All the experiments had 3 replications, each one of 10 leaves. The study involved some valuable essential oil rose cultivars, growing on the collection plots of NBG-NSC: Raduga, Festivalnaya, Lany, Michurinka. Such viral damages as chlorotic spots, mosaic, leaf blade folding and deformation were visually noticed in plants.

With the aim of virus elimination the above described cultivars were introduced to *in vitro* culture. Meristems 0.2-0.4 mm size were isolated from vegetative buds under a stereoscopic binocular microscope Nikon SMZ 745T (Japan) and introduced into a laminar flow cabinet SC2-4A1 (ESCO, Singapore) on the modified Murashige and Skooge medium (1962), supplemented with 0.1 mg/l α-naphthylacetic acid (NAA), 0.5 mg/l gibberellic acid (GA), 0.5-1.5 mg/l 6-benziaminopurina (BAP), 30 g/l sucrose and 8 g/l agar. For *in vitro* chemotherapy 10-25 mg/l virocid 1-beta-D-ribofuranosyl-1H-1,2,4-triazole-carboxamide (ribavirin, "Sigma", USA) was added. The explants were cultured under controlled climatic conditions (growth room): temperature 24±1°C, cool white light intensity 25.0-37.5 μmol m^{-2} s^{-1} and 16-hour photoperiod. Plant samples for analyses were collected after 9-12 months of culture. Each treatment included 5 vessels with 3 regenerant seach.

Total water content of leaves determined by the method of thermal drying at 105°C, water fractional composition determined with Marinchik-Gusev method, water-holding capacity and resistance to dehydration found by the methods of Eremeev and Lischuk[8] and water scarcity defined with the method by Kouchnirenko, Kurchatov and Kryukovoy[9] were used as physiological criteria characterizing plant attitude to poor water supply and high temperatures. Climate characteristics at the time studies are presented according to Agrometeostation "Nikita Garden" (village Nikita, Yalta, Russian Federation).

Parameters of photosynthetic activity were measured with portable fluorometer "Florotest" (V.M. Glushkov Institute of Cybernetics of NAS of Ukraine, 2010). Leaves were previously adapted to the dark for 8 minutes. The optimal extrapolation duration corresponded to the time of Kautsky curves output of on stabilization plateau (3 minutes). The LED had maximum radiation intensity at λ = 470±20 nm. The following parameters were fixed in the experiment: the initial level of fluorescence after irradiation (F_0), maximum (F_m) and stationary (F_{st}) fluorescence values after light adaptation. Variable fluorescence, viability index and photosynthetic activity were calculated[10, 11].

Biochemical parameters were determined by common techniques. Proline content was determined by the modified Chinarda method using ninhydrin reagent[12], the amount of phenolic compounds – by photometric method with Folin-Ciocalteu reagent[13], ascorbic acid by iodometric titration[14]. Catalase (EC 1.11.1.6) activity was determined by titration method[15], polyphenol oxidase (EC 1.14.18.1) activity – by colorimetric method in the presence of catechol and p-fenilendiamina[16], superoxide dismutase (EC 1.15.1.1) activity–with kvercetin oxidation reaction[17].

Results and Discussion

Under the conditions of field growth on the collection plots of NBG-NSC during the optimal growing season (May-June) water content in the leaves essential oil rose ranged from 63 to 65%, including the bound water part 87-92% of its total (Table 1). The level of water deficit in the first decade of June (average air temperature 21.9°C, minimum relative humidity – 41%) ranged from 15 to 18% in the cultivars Raduga, Festivalnaya and Michurinka; this index was higher in the cultivar Lany (23%). Data obtained in the process of diagnosing the functional state of the leaves through the fluorescence induction of the exposed leaf, demonstrated the absence of photosynthetic apparatus disturbances (relative photosynthetic activity in field studies ranged from 0.66 (‘Michurinka’) to 0.79 a.u. (‘Festivalnaya’). The water holding capacity of leaf tissue was the highest in cultivars Festivalnaya (after 8 hours leaves lose 24-26% of water, after 24 hours – 44-48%) and ‘Raduga’ (28-34% and 39-50%). Minimum water holding capacity was in the cultivars Lany and Michurinka.

Table 1: The water regime parameters in the leaves of essential oil rose, in various vegetation conditions

Cultivar, vegetation conditions		Total water content, %	Free water part, %	Bound water part, %	Water deficite, %	Water holding capacity of leaf tissues (% of water loss from the full saturation)	
						after 8 hours	after 24 hours
Raduga	1*	63.80±3.73	12.13	87.87	15.44±2.14	31.43	49.14
	2	52.73±2.05	8.88	91.12	23.92±0.72	29.34	43.92
Festivalnaya	1	64.54±2.47	10.96	89.04	17.89±1.80	25.57	47.59
	2	55.76±1.37	9.50	90.50	20.59±3.71	28.80	44.41
Lany	1	65.10±4.52	7.57	92.43	22.36±1.15	37.00	50.33
	2	53.79±1.23	13.59	86.41	24.35±0.86	31.04	43.79
Michurinka	1	63.64±4.57	11.31	88.69	17.04±3.05	31.37	49.51
	2	49.91±3.76	11.68	88.32	26.96±1.56	34.98	47.43

* 1 – optimal growing conditions (the first decade of June); 2 – extreme conditions (the second decade of August).

During the growing season under the hydrothermal stress (second decade of August: average air temperature 26.9°C, maximum – up to 31.7°C, temperature on the ground surface up to 57.8°C, the relative humidity dropped to 47%) water content of leaves in all studied cultivars decreased to 46-64%, that was 9-13% lower the values in the optimal growing conditions. At the same time, the part of bound water, especially in the cultivars Raduga and Festivalnaya (Table. 1). Water deficit increased in all cultivars to 21-27%. Fotosistem-2 functions were not violated, the variable fluorescence had 35 - 40% increase, relative FA was 0.76 - 0.81 a.u. These data let us to suggest that noon increase of water deficit was compensated by renewal of the turgescent state at night.

Rapid water loss under wilting of exposed leaves occurred after 4 hours in cultivars Michurinka (21%) and Lany (18%), and after 6 hours of wilting in the cultivars Raduga and Festivalnaya to the level of 20-23%. During the growing season we also observed increase of water holding capacity of leaf tissues: within 24 hours the water loss was 42-48%. Under significant water loss (25-30%) in the leaves of the cultivar Festivalnaya inhibition of photosynthetic function ($(F_m$-$F_{st})/F_m$=0.75 a.u.) wasn`t observed. In the cultivar Michurinka leaves under the water loss of 15% disturbances in photosystem-2 ($(F_m$-$F_{st})/F_m$=0.54 a.u.) and at the level of light-harvesting complexes of the photosystem inhibition was noted after 8 hours wilting (water loss 35%).

Thus, on the base of essential oil rose water regime analyses *in situ* cultivars Festivalnaya and Raduga could be marked as plants with higher ecological and physiological potential in arid conditions.

During *in vitro* culture on various media and of different terms hydration level of regenerants` vegetative organs in essential oil rose was high: the total water content in leaf tissue varied from 87 to 89% of water to the wet weight (Table 2.). Since the behavior of water in the cell is not determined by its amount but its activity and thermodynamic state[9], we studied the water fractional composition in the cultured plants. It should be borne in mind that hydrated water has an important function in the water transport through the membrane. Under positive hydration solutes limited water mobility, while in negative one they enhance it. The part of "free" water (capillary and osmotic-absorbed) was up to 26-29% of its total content. Accordingly, the part of water fraction, ordered with low- and high-molecular compounds, reached 61-74% of the total content. High water holding capacity *in vitro* was typical for the cultivar Raduga leaves.

Table 2: Characteristics of the water regime and photosynthetic activity in essential oil rose regenerates cultured *in vitro*

Cultivar	Water content of the leaf tissues (M±SE)			Indexes of chlorophyll fluorescence induction, a.u.			
	Total water content, %	Free water part, %	Bound water part, %	F_v	$(F_m-F_{st})/F_m$	F_m/F_{st}	F_v/F_m
Raduga	86.67±2.86	25.64±3.84	74.04±3.68	472	0.52	2.07	0.68
Festivalnaya	82.50±3.15	29.15±2.05	69.86±4.92	464	0.51	2.05	0.69
Lany	88.89±7.21	29.05±3.65	69.95±5.12	384	0.61	2.57	0.53
Michurinka	85.06±4.84	36.19±3.85	61.54±5.02	469	0.55	2.23	0.60

Values of the main fluorescence parameters of the studied plants are presented in Table 2. Strengthening of the fluorescent signal from F_0 to F_m is reflected in the values of variable fluorescence (F_v). According to its ration to the maximum level (F_m) photoinhibition at the level of light-harvesting complexes of photosystem-2 could be estimated. In studied cultivars this index was in the normal range (from 0.53 a.u. in the cultivar Lany to 0.68 in the cultivar Raduga). Some authors [10, 11] supposed connections between the IPC kinetics and photosynthetic assimilation of carbon dioxide. So, photosynthetic activity can be evaluated with the expression $(F_m- F_{st})/F_m$. This index is normal in Lany cultivar, and a little lower in the cultivars Raduga and Festivalnaya. Viability index – F_m/F_{st} was 2.05-2.57 a.u. All these data suggest normal functional state of photosynthetic apparatus in the studied rose cultivars *in vitro*. Lack of photoinhibition indexes and active work of photosynthetic apparatus in regenerants let us to suppose high adaptive potential of the studied cultivars in postaseptic culture conditions.

At the same time for guarantee selection of high-resistant essential oil rose cultivars information about the content of protective compounds of different chemical nature under different conditions of plant cultivation is necessary. Such studies have been made previously on *Canna* x *hybrida* hort. cultivars and let us to confirm their high degree of adaptability to hydrothermal stress and to identify structural, functional and biochemical features in Canna with symptoms of viral diseases[18].

In field growing conditions during the optimum growing season plants of all studied essential oil rose cultivars had a high content of phenolic compounds (1204-1399 mg/100 g), particularly flavanols (988-1202 mg/100 g) (Table 3).

Potentially, the high content of these compounds in studied plants and visually infections damages confirms the presence of viruses in investigated cultures. Being accumulated in plants, phenolic compounds can inhibit growth and development of pathogen microorganisms, production and activity of their metabolits[19].

Concentration of ascorbic acid, depending on the cultivar, varied in the range of 24.99 mg/100 g (‘Michurinka’) up to 47.87 mg/100 g (‘Lany’). Proline content in studied rose cultivars ranged from 9.42 mg/100 g (‘Festivalnaya’) to 12.95 mg/100 g (‘Michurinka’).

During vegetation period under hydrothermal stress the total content of phenolic compounds, particularly flavanols, increased in studied plants, except the cultivar Michurinka. Despite the slower metabolism of phenolic compounds in the absence of intensive plants` growth and development, increased synthesis of these compounds may correspond to plant response over stress caused by overheating. Concentrations of ascorbic acid and proline in this vegetation period varied ambiguous: in the cultivars Festivalnaya and Raduga proline content decreased, whereas ascorbic acid – increased. In the cultivars Lany and Michurinka the opposite trend was noted. The reasons for these differences are specific reaction of plants to the temperature stress, i.e. primary accumulation of specific protective compounds, and physiological features of plant development in this period.

Table 3: The content of some protective compounds in essential oil rose under different culture conditions

Cultivar	Conditions	Content, mg/100 g			
		Proline	Ascorbic acid	Phenolic compounds	Flavanols
Festivalnaya	*in situ* 1*	9.42±0.26	31.68±0.95	1204±34	1001±30
	in situ 2	6.28±0.19	55.00±1.63	1469±44	1365±38
	in vitro	10.99±0.30	29.92±0.81	1150±33	52.0±1.5
Lany	*in situ* 1	12.95±0.38	47.87±1.43	1305±34	1105±31
	in situ 2	34.54±0.83	39.16±0.98	1515±41	1404±42
	in vitro	16.48±0.47	42.24±1.26	1134±32	26.0±0.8
Raduga	*in situ* 1	10.99±0.32	45.06±1.35	1243±37	988±28
	in situ 2	9.42±0.28	71.28±1.85	1399±40	1183±34
	in vitro	19.62±0.57	31.68±0.95	1026±30	23.4±0.7
Michurinka	*in situ* 1	12.17±0.35	24.99±0.70	1399±41	1202±35
	in situ 2	40.03±1.12	19.80±0.46	1352±38	1131±33
	in vitro	13.34±0.36	21.12±0.63	1958±51	46.8±1.4

* 1 – optimum conditions of vegetation (the first decade of June); 2 – extreme conditions (the second decade of August).

Nevertheless, proline that has osmoregulatory functions[20, 21], may interact with surface proteins hydrophilic residues, increasing their solubility and protecting against denaturation. As a result, the cells retain more water that enhances the vitality of plants under high temperature conditions. At the same time biosynthesis of ascorbic acid associated with plant photosynthesis and respiration activates[22].

Phenolic compounds` content in plants cultured *in vitro* was quite high (1056-1958 mg/100 g). The increased accumulation of phenolic compounds demonstrated high adaptability to the growth conditions and resistance to unfavourable environmental conditions. Thus flavonol concentration in plants cultured *in vitro* was significantly lower than in intact plants – 23.4-52.0 mg/100g (maximum in 'Festivalnaya'). Ascorbic acid concentration in plants *in vitro* was lower than in intact plants have not been subjected to overheating.

On the contrary, proline content *in vitro* was higher than in intact plants have not been subjected to thermal stress, up to 10.99-19.62 mg/100 g (maximum in 'Raduga'). High amount of this compound is probably, caused with plants` growth and metabolism, as well as nutrients, vitamins and growth regulators level in the culture medium.

Maximum rates of catalase, superoxide dismutase and polyphenoloxidase activity were found in rose cultivars Festivalnaya, Lany, Raduga, cultured *in vitro* (Table 4).

Table 4: Activity of redox enzymes in rose cultivars

Cultivar	Conditions	Catalase activity, g O_2/g·min	SOD activity, a.u./g	PPO activity, a.u./g·sec
Festivalnaya	*in situ*1*	9.26±0.27	8.88±0.25	0.092±0.002
	in situ 2	8.93±0.25	10.52±0.30	0.111±0.003
	in vitro	10.91±0.32	13.60±0.38	0.183±0.005
Lany	*in situ* 1	14.49±0.40	6.26±0.19	0.071±0.002
	in situ 2	14.03±0.41	7.58±0.22	0.079±0.002
	in vitro	15.02±0.42	10.12±0.28	0.103±0.003
Raduga	*in situ* 1	15.31±0.44	2.06±0.06	0.094±0.003
	in situ 2	14.69±0.42	2.89±0.08	0.111±0.004
	in vitro	16.01±0.45	4.28±0.12	0.139±0.004
Michurinka	*in situ*1	11.56±0.32	11.72±0.32	0.096±0.003
	in situ 2	11.90±0.35	11.59±0.32	0.100±0.003
	in vitro	11.26±0.30	12.36±0.36	0.061±0.002

* 1 – optimal vegetation conditions (the first decade of June); 2 – extreme conditions (the second decade of August).

In plants of studied cultivars grown *in situ*, catalase activity decreased along with increase of hydrothermal stress. Superoxide dismutase and polyphenol oxidase activity decreased during the optimum vegetation period and increased under the influence of stress factor. It should be noted that the increase in polyphenol oxidase activity was observed with increased content of phenolic compounds, particularly flavonoids. Activation of SOD and polyphenol oxidase is an important factor in preventing the of oxidative stress development in studied rose cultivars. Increasing of those enzymes activity is favorable to rapid adaptation of plants to hydrothermal stress.

In plants, of the rose cultivar Michurinka grown *in vitro* and *in situ* catalase activity changed slightly, and under the stress strengthening it slightly increased. Also, with the stress factors strengthening polyphenol oxidase activity slightly increased. At the same time, superoxide dismutase activity decreased. Such changes of enzyme activity appeared to be associated with low resistance of Michurinka cultivar to adverse environmental factors.

Based on experiment results we can conclude the high degree of adaptation of different essential oil rose cultivars both in controlled laboratory conditions (*in vitro*) and grown in the open field (*in situ*). These features were provided by xeromorphic structure of vegetative organs, high water holding capacity of leaf tissues due to the bound water fraction. In the formation of the essential oil rose plant protective response to hydrothermal stress and further adaptation response accumulation of protective compounds (free proline, phenolic compounds, and ascorbic acid) is significant. At the same time in different cultivars of essential oil rose amount and rate of protective compounds accumulation differed. The impact of hydrothermal stress on the cultivars Festivalnaya, Lany and Raduga led to decrease in catalase activity and increased superoxide dismutase and polyphenol oxidase activity. These changes are corresponds to the plasticity of investigated cultivars and thus their ability to rapid adaptation to changing environmental conditions. Based on the investigation results we can recommend rose cultivars Festivalnaya and Raduga for general use in the essential oil industry in regions with a semi-arid climate.

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