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ADAPTIVE CAPACITY OF SOME LAVENDER AND LAVANDIN CULTIVARS*IN VITRO* AND *IN SITU*

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ABSTRACT
Lavandula angustifolia Mill. and (*Lavandulaxintermedia*EmericexLoisel) are promising fragrant plants with medicinal, aromatic and ornamental properties. Since the collection plantations of these crops are very damaged with viral pathogens and there is lack of seed propagation in valuable cultivars 'Belyanka', 'Record' (lavender) and 'Rabat', 'Snezhnyi Bars' (lavandin), were introduced *in vitro*. Chemotherapy was used for cleaning up. Regenerants were cultured (4-5 months) on MS medium with 0. 3 mg L^- Kinetin, 0. 025 mg L^- NAA and 0. 25 mg L^- GA_3 at 25±1°C under 16-h photoperiod. Intact plants were studied during the growing season. In order to reveal plants‘ biotechnological and genetic capacity some biochemical stress indicators, indexes of photosynthetic activity and water regime were identified. Under the open field cultivation, tested plants were rich in ascorbic acid, phenolic compounds, and redox enzymes (catalase, polyphenol oxidase, superoxide dismutase) were active. Leaf tissue hydration was 56-62%, with greater part of bound water. Photosynthetic activity was reduced only in the samples with visible damages with viral pathogens. In plants cultured *in vitro*, amount of ascorbic acid and phenolic compounds were lower, so as enzymatic activity and proline concentration were higher than in intact plants. The rate of hydration was high (70-77%), with the same trend of water fractional composition. Photosynthetic activity and vitality index indicated no photoinhibition. It was found out the lavandin cultivars had better capacity for a wide use under various culture conditions.

Keywords: *Lavandula sp, tissue culture, biochemical indicators, photosynthetic activity, water regime.*

INTRODUCTION
Lavender (*Lavandula* L.) is a valuable essential oil, aromatic, decorative and medicinal culture. The main cultivated species are *Lavandula angustifolia* Mill. and lavandin (*L. hybrida* Rev. or *Lavandulaxintermedia*EmericexLoisel). Lavender and Lavandule plants contain valuable essential oil, which is used in medicine,

perfume, cosmetics and food industry (Libus et al., 2004). Phenolic compounds, which have a wide range of physiological activities, were also identified in plant feedstock (Torras-Claveria et al., 2007).

Medical feedstock is the entire aboveground mass of plants. Traditionally, these crops are propagated by seeds (*L. angustifolia*) or vegetative (*L. angustifolia* and *L. hybrida*). In recent years, high degree of viral pathogens damages was noticed for cultivated plants. High quality planting material may be obtained by cleaning up *in vitro*. Besides, biotechnological methods enable to produce a large number of genetically homogeneous virus-free plants (Mitrofanova et al, 2014; Gonçalves and Romano, 2013). Plants damaged with viral pathogens are exposed to stress. One of the earliest responses to the stress effect is active forms of oxygen (AFO) formation and they violate many processes in the cell and its structure. To prevent such violations antioxidant systems including low molecular weight protective compounds and specific antioxidant enzymes are present in cells. The study of the antioxidant systems functioning is important for predicting plant adaptive capacity (Mittler, 2002; Mullineaux and Baker, 2010). The sensitive parameter for changes in plant functional state is also photosynthetic activity and the viability index (Byron et al, 2000; Stirbet and Govindjee, 2011).

Thus, the aim of the work was to determine physiological and biochemical changes in vegetative organs of valuable lavender and Lavandula cultivars in plants with symptoms of viral diseases and after cleaning up *in vitro*.

MATERIAL AND METHODS

The objects of this study were valuable cultivars of lavender (Belyanka, Record) and lavandula (Rabat, Snezhnyi Bars) from the collection of Nikita Botanical Gardens. The researches of intact plants were carried out during the phenophase "technical maturity" (the second and third decade of July 2015). For the studies plants without visual symptoms of viral diseases and damaged ones were selected. Apical meristems of axillary buds were introduced *in vitro*. Chemotherapy *in vitro* was used for cleaning up.

Regenerants were cultured for 4-5 months on the modified MS medium with 0. 3 mg l^- Kinetin, 0. 025 mg l^- NAA and 0, 25 mg l^- GA_3. Explants in the culture vessels were kept in a growth chamber at 25 ± 1°C under 16-h photoperiod supplied by cool-white fluorescent lamps giving 37. 5 µmol m^- s^-. The callus formed 2-5 microshoots, 23-82 mm height and each microshoot produced from 10 to 26 lanceolate leaves, 9-15 mm length.

Biochemical parameters were determined by standard methods: the content of proline due to modified procedure by Chinarda using ninhydrin reagent (Andryushchenko et al.,1981), the amount of phenolic substances by photometric method using Folin-Ciocalteu reagent (Gerzhikova, 2002), ascorbic acid by iodometric titration (Richter, 1999). The activity of catalase (EC 1. 11. 1. 6) was determined titrimetrically (Voskresenskayaetal.,2006), polyphenol oxidase (EC 1. 14. 18. 1) - in the presence of catechol and p-phenylenediamine (Ermakov, 1987), superoxide dismutase (EC 1. 15. 1. 1) – due to quercetin oxidation reaction

(Kostyuk et al., 1990). Such physiological criteria characterizing the water regime as the total water content in leaves, water fractional composition and the water deficit under the cultivation in the field were used (Ross, 2012). Weather data of the study period are presented according to the observations of agrometeorological station "Nikita Garden": the average air temperature in the period of water deficit studies was 22. 2˚C, the maximum air temperature – 32. 4˚C, minimum relative humidity – 39%. The basic amount of precipitation fell in the beginning of July; their sum for the month was 15. 6 mm or 50% of the norm. In late July, stocks of productive soil moisture at the depth of the lavender root layer (0-20 cm) were 40 mm. The parameters of photosynthetic activity were measured with a portable fluorometer "Florotest" (Ukraine, 2010). During the experiments the following indexes of Kautsky fluorescence induction kinetics were registrated: the initial fluorescence (F_0), the maximum (Fm) and stationary (Fst) fluorescence values after dark adaptation. Viability index and photosynthetic activity were calculated (Bajron et al., 2000).

RESULTS AND DISCUSSION

To evaluate the adaptive capacity of plants requires data about protective compounds of various chemical origin are needed. Proline is among those compounds and it is a source of energy, carbon and nitrogen under the lack of resources and reduce of synthesis enzyme activity caused by stress (Kavi Kishor et al., 2005). Other proline functions including osmoregulation and participate in gene expression were found out (Lyers and Caplan, 1998).

In the conditions of the open field growing for lavender and Lavandula plants withoutvisual symptoms of viral diseases proline content was the highest and it decreased slightly (to 29%) in the plants with symptoms of viral diseases (Table 1). Perhaps, proline reduction in the plants with viral symptoms was due to proline synthesis slowdown in response to stress and its consumption for engagement with surface hydrophilic residues of proteins to increase their solubility and protection from denaturation. In regenerants under *in vitro* conditions, proline concentration was higher than in intact plants, despite the high water content in tissues. This suggests that free proline can influence the processes of cell growth and differentiation in Lavender and Lavandula.

Phenolic substances are also among the main plant protective compounds. They are involved in the basic processes of plant cell activity: photosynthesis, respiration, as well as stress protection (Zaprometov, 1993). Plant response to viral infection is the increase of phenolic compounds content (Dikilitas et al., 2011).

Lavender and Lavandula intact plants without visual viral diseases symptoms accumulated maximum amount of phenolic compounds. Together with viral diseases symptoms appearance phenolics content reduced (to 14-31%), which is probably corresponds to the specific reactions of these cultures for viral infection, i. e. preferred accumulation of protective compounds of the other chemical origin. Antioxidant protection of that type plants can be carried out due to the presence of high-molecular antioxidants and volatile compounds.

Considering that influence of adverse factors on plant resulted in oxidative stress ascorbic acid is under the interest as a protective compound. It is the most common antioxidant in plants. It is known that this compound is a regulator of cell growth and cofactor for many enzymes and it is involved in photosynthesis, respiration and growth of plants (Smirnoff, 2000).

In intact plants without visual viral diseases symptoms concentration of ascorbic acid was the highest and it slightly decreased (by 15-29%) together with viral diseases symptoms appearance. The exception was Lavandula cultivar Snezhnyi Bars, in which the changes in ascorbic acid content under the appearance of viral diseases symptoms did not demonstrate significant difference.

In plants under controlled conditions *in vitro* concentration of phenolic compounds and ascorbic acid were lower than in intact plants, due to high water content in tissues and absence of stress factors.

Table 1. Biochemical indexes of lavender and Lavandula cultivars

Cultivars		Proline, μg g^{-}	Ascorbic acid, mg 100 g^{-}	Phenols, mg 100 g^{-}
Belyanka	*in situ*	7. 69±0. 23	20. 06±0. 58	1033±26
	*in situ**	5. 49±0. 16	18. 04±0. 52	785±23
	in vitro	8. 24±0. 24	5. 61±0. 16	645±17
Record	*in situ*	12. 95±0. 37	18. 92±0. 54	1181±31
	*in situ**	10. 99±0. 32	15. 05±0. 44	816±24
	in vitro	33. 75±0. 99	5. 94±0. 17	490±14
Rabat	*in situ*	6. 67±0. 20	19. 14±0. 55	1305±34
	*in situ**	5. 49±0. 14	15. 40±0. 45	1119±30
	in vitro	35. 72±1. 04	4. 95±0. 13	668±19
Snezhnyi Bars	*in situ*	21. 59±0. 63	14. 96±0. 44	1492±40
	*in situ**	18. 05±0. 53	15. 62±0. 46	1165±32
	in vitro	35. 32±1. 05	5. 98±0. 17	777±22

* - plants with visual symptoms of viral diseases

The results presented in Table 2 demonstrate that in the studies of redox enzymes in and lavender and Lavandula cultivars it was determined that plants without visual symptoms of viral diseases are characterized by maximum values of catalase, polyphenol oxidase and superoxide dismutase activities.

In plants with symptoms of viral infection activity of all studied enzymes decreased: catalase activity by 10-56%, SOD activity - by 5-26%, PPO activity - by 22-60, suppose that is associated with reduced antioxidant status of plants due to excessive accumulation of the active forms of oxygen. The greatest decrease in catalase and PPO activity together with poor changes of SOD activity was observed in infected Lavandula plants cultivar Rabat.

Table 2. Redox enzymes activity in lavender and lavandin cultivars

Cultivars		Catalase activity, g $O_2 g^- min^-$	SOD activity, a. u g^-	PPO activity, a. u. $g^- \cdot s^-$
Record	*in situ*	18. 13±0. 45	13. 60±0. 33	0. 628±0. 016
	*in situ**	15. 45±0. 39	11. 28±0. 27	0. 472±0. 012
	in vitro	6. 80±0. 16	6. 12±0. 20	0. 101±0. 003
Belyanka	*in situ*	30. 68±0. 87	12. 98±0. 32	0. 524±0. 13
	*in situ**	25. 50±0. 64	10. 83±0. 27	0. 378±0. 009
	in vitro	7. 65±0. 19	5. 62±0. 14	0. 103±0. 002
Snezhnyi Bars	*in situ*	36. 97±0. 92	14. 82±0. 38	0, 377±0. 008
	*in situ**	36. 12±0. 90	10. 83±0. 26	0, 294±0. 007
	in vitro	2. 98±0. 08	10. 48±0. 28	0. 124±0. 004
Rabat	*in situ*	31. 45±0. 77	12. 55±0. 32	0. 600±0. 015
	*in situ**	13. 74±0. 43	11. 93±0. 30	0. 238±0. 007
	in vitro	3. 68±0. 09	12. 43±0. 31	0. 112±0. 003

Under *in vitro* culture catalase activity in Lavender cultivars was higher than that in Lavandula ones. At the same time, SOD and PPO activity in lavender *in vitro* had lower values than the in Lavandula. Comparative analysis of enzymes activity in intact plants without viral diseases symptoms and plants cleaned up *in vitro* demonstrated that minimum values of catalase and polyphenol oxidase activity are characteristics of Lavender and Lavandula cultivars grown *in vitro*. Reduction of enzyme activity corresponds to the high water content in tissues, low content of ascorbic acid and phenolic compounds, as well as the absence of stress factors. SOD activity in Lavandula cultivars *in vitro* was at the same level with the healthy plants grown *in situ*, and in Lavender cultivars *in vitro* it was 50% lower than in healthy intact plants.

Table 3. Indexes of the water regime and the relative quantum efficiency of photosystem-2 in Lavender and Lavandula cultivars

Indexes		Cultivars			
		Belyanka	Record	Rabat	Snezhnyi Bars
Total water content, %	*in situ*	61. 1 ± 3. 0	57. 9 ± 2. 5	56. 3 ± 4. 8	62. 3 ± 2. 1
	*in situ**	59. 1 ± 2. 9	58. 1 ± 2. 8	70. 8 ± 1. 9	58. 2 ± 2. 5
	in vitro	76. 1 ± 3. 3	72. 3 ± 2. 9	77. 0 ± 2. 5	74. 4 ± 3. 2
Bound water fraction, % of total water content	*in situ*	78. 3 ± 4. 9	90. 6 ± 3. 5	82. 1 ± 4. 3	93. 2 ± 1. 3
	*in situ**	84. 9 ± 2. 3	87. 4 ± 1. 9	79. 3 ± 5. 0	86. 5 ± 4. 3
	in vitro	69. 5 ± 4. 1	58. 1 ± 2. 2	68. 3 ± 4. 8	49. 4 ± 6. 1
Plant-water deficit, %	*in situ*	26. 9 ± 1. 4	24. 8 ± 2. 9	23. 1 ± 2. 9	29. 1 ± 1. 2
	*in situ**	23. 5 ± 2. 9	38. 2 ± 4. 1	26. 4 ± 1. 4	25. 3± 1. 8
Relative photosynthetic activity$(F_m\text{-}F_{st})/ F_m$	*in situ*	0. 68 ± 0. 09	0. 70 ± 0. 05	0. 75 ± 0. 10	0. 71 ± 0. 05
	*in situ**	0. 62 ± 0. 07	0. 52 ± 0. 08	0. 73 ± 0. 12	0. 68 ± 0. 02
	in vitro	0. 28±0. 10	0. 45±0. 05	0. 55±0. 08	0. 45±0. 09
Viability index F_m/F_{st}	*in situ*	2. 61 ± 0. 50	2. 51 ±0. 61	3. 18 ± 0. 52	2. 94 ± 0. 70
	*in situ**	1. 94 ± 0. 31	1. 82 ± 0. 29	2. 73 ± 0. 68	2. 85 ± 0. 32
	in vitro	1. 41±0. 03	1. 71±0. 12	2. 36±0. 37	2. 00±0. 36

Under the open field growth conditions water content in leaves was 56-62% (Table 3) and the part of bound water was from 79 to 93% of the total water content. After a long period (18 days) period without precipitation total water content in vegetative organs decreased, while the part of bound water increased. The maximum water-holding capacity was characteristic of the vegetative organs tissue in the cultivars Snezhnyi Bars and Record due to the fraction of bound water. Studied parameters did not reveal any significant differences between the plants with visual symptoms of viral diseases and ones without symptoms.

The level of leaf-water deficit was 23-29% in the cultivars Rabat, Belyanka and Snezhnyi Bars. The maximum (38%) leaf-water deficit was noticed in Record cultivar plants with the symptoms of viral pathogens damages. We did not notice significant differences between the affected and unaffected plants in other studied cultivars. Changes in the water regime and the degree of cultivar susceptibility affected largely on the photosynthetic activity of Lavender cultivars. They demonstrated decrease of the relative quantum efficiency of photosystem 2, photochemistry and efficiency of the energy capture with open reaction centers. Viability index was in the normal, but it was lower in Lavender cultivars. Photoinhibition expressed in plants with visual symptoms of viral diseases. The observed effect of changes in the photosynthetic apparatus activity together with visual damages of vegetative organs in the studied plants are in agreement with the conclusions by S. Nogues and L. Alegre (2002).

Leaf water content in *in vitro* regenerants was higher in Lavandula plants (74-77%) and no significant differences in this parameter was not revealed between the cultivars. However, the lowest variability of water content in regenerants during the culture and maximum ratio bound/free water fraction let us to select the cultivars Rabat and Record. The high photosynthetic activity of leaves in regenerated plantlets was found. Viability index was normal for plants grown under controlled conditions *in vitro* and the same was in plants grown at a relatively heterotrophic nutrition type. According to these indexes, better functional state was characteristic of Lavandula cultivars.

Functional state indexes of the studied plants *in vitro* demonstrated no photoinhibition and normal activity of photosystems at both light harvesting complexes and at the time of electron donors' oxidation in the reaction center of photosystem 2.

CONCLUSIONS

It was found that in the open field growth conditions content of proline, phenolic compounds, ascorbic acid and the activity of catalase, superoxide dismutase and polyphenol oxidase were maximum in Lavender and Lavandula symptomless and they decreased in plants with symptoms of viral diseases. According to those parameters, no significant differences between Lavender and Lavandula cultivars were revealed. Changes in the water regime of the cultivars and the degree of their susceptibility affected largely on the photosynthetic activity in Lavender cultivars.

In plants under *in vitro* conditions proline concentration was higher and the content of phenolic compounds, ascorbic acid and the enzymatic activity was lower than in intact plants. Photosynthetic activity and vitality index demonstrated no photoinhibition.
It was revealed that studied Lavandula cultivars have greater plasticity and capacity for growing *in vitro* and *in situ*.

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