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The effect of negative air temperatures and humidity on the photosynthetic apparatus functioning in some species of *Oleaceae* family

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Abstract. The characteristics of the photosynthetic apparatus functioning in the leaves of some evergreen species of *Oleaceae* family depending on the cold periods` weather conditions on the southern coast of Crimea are presented in this work. It has been revealed that changes in PSII are species-specific. Low air humidity in combination with negative air temperature promotes a significant decrease in frost resistance in the studied *Olea* and *Osmantus* genotypes. However, in *Olea europea* genotypes, changes in the PSII parameters under the simulation of winter desiccation approximated sublethal limits. The photosynthetic apparatus in *L. lucidum* species was more sensitive to a decrease in air temperature under conditions of relatively high air humidity. The development of the stress state in this species was accompanied by an increase in energy losses in the process of energy transfer from light-harvesting complexes. With the onset of frosty weather, a significant decrease in the chlorophyll a/b ratio was noted in all the studied genotypes. After the stress pressure, *Olea europea* cultivars and *Osmantus* spp. showed an increase in chlorophyll amount, in contrast to *L. lucidum*, in which the ratio of photosynthetic pigments remained low.

1 Introduction

One of the most important problems of horticulture in the modern world is the search for plant genotypes with complex resistance to stress factors that limit the cultivation and use of valuable plant species and cultivars in human economic activity. On the backdrop of global climate change, this issue is becoming extremely relevant, especially for subtropical regions. At present, in the southern regions, the frequency of damages to the introduced subtropical plant species has increased significantly, due to more frequent abrupt fluctuations in air temperature during cold periods and an increased probability of extreme hydrometeorological events [1]. Evergreen species of the Oleaceae family are important sources for improving the range of ornamental plants for creating comfortable urban landscapes. *Olea europea* genotypes, for which the Southern Coast of Crimea (SCC) is the northern border of cultivation, in addition to decorative properties, are valuable agricultural plants. In recent years, droughts have become a frequent and prolonged phenomenon in the

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Southern Coast of Crimea, and a decrease in air temperature to negative values in the winter seasons is often accompanied by low relative air humidity and gusty winds, which adversely affect the implementation of adaptation processes in a number of introduced species. It has been previously shown that a high level of water deficiency in the leaves of *Olea europea* cultivars contributes to a decrease in their frost resistance [2]. Maintaining the viability and productivity of plants under conditions when two factors are beyond the optimum often depends on their ability to function under stress and not so much on their potential resistance. Analysis of the scientific literature showed that studies of the frost resistance in *Olea europea* varieties and cultivars had been carried out mainly in relation to negative temperatures, without considering the accompanying meteorological factors [3,4].

The goal of the presented research was to reveal the special features of the photosynthetic apparatus functioning (chlorophylls accumulation and PS II parameters) during the cold periods on the Southern coast of Crimea, and the effect of air humidity on the photosynthesis processes under the negative temperatures.

2 Objects and methods of research

The objects of the research were the leaf apparatus of some evergreen species from Oleaceae family, characterized with various frost resistance: *Olea europea* (frost-resistant cultivar Nikitskaya and nonresistant cultivar Ascoliano), *Osmantus* species (relatively resistant species *O.×fortunei* and sensitive to negative temperatures *O. fragrans*), and frost-resistant species *Ligustrum lucidum*. Changes in chlorophyll fluorescence induction (CFI) parameters were studied with a chronofluorimeter “Floratest”. The following parameters were analyzed: F_0 - initial fluorescence; F_m - maximum fluorescence; F_{pl} - fluorescence at the time of its temporary slowdown; F_s - stationary fluorescence. Based on the recorded parameters we calculated variable fluorescence (F_v=F_m-F_0) ; the number of unreduced Q_a in PSII reaction centers, (F_{pl}-F_0)/F_v; the ratio between the rate constants of photochemical and non-photochemical deactivation in PSII, F_v/F_0, as well as the fluorescence decay coefficient (viability index) – F_m/F_{st} and the efficiency of the light phase of photosynthesis, F_v/F_m [5,6]. Measurements were taken during cold periods, monthly, taking into account changes in hydrothermal conditions. Experiments on the air humidity effect under the pressure of negative temperatures were carried out in a Binder climate chamber. Variant 1 - air temperature -8°C for 6 hours, at a relative humidity of 60%. Variant 2 - air temperature -8°C for 6 hours, at a relative humidity of 30%. The control was leafy shoots maintained at a temperature of +8°C ... +10°C for 12 hours. All the experiments had 3 replications. The data were analyzed with MS Excel 2007. The significance of differences between the variants was assessed by Student's t-test at a 5% significance level. The tables and graphs show the mean values with the standard error.

The total chlorophylls amount was quantified in the leaves of the current generation during the cold periods (November - March) 2019-2020 and 2020-2021 by the spectrophotometric method [7].

3 Results and Discussion

During the cold periods of 2018-2021, taking into account hydrothermal conditions, studies of photosynthetic activity were carried out in some Oleaceae species, characterized by different frost resistance. According to the data of the agrometeorological station "Nikitsky Sad", the weather conditions of the cold period in 2018-2019 on the Southern Coast of Crimea were characterized by high cyclonic activity and large amount of precipitation. In November, December and January, the amount of precipitation was 150% of the monthly norm. Average monthly temperatures exceeded the norm by 1.6…2.5°C. The minimum temperature did not fall below -1.4°C. It was found out that at this time, in the studied Oleaceae species, the maximum photochemical activity of PSII changed insignificantly. Cold period 2019-2020 was drier. In November, the reserves of productive moisture in the soil were less the optimum (29-40% field capacity (FC) ~~НВ~~). In December and January, precipitation was only 62% of the norm. A significant decrease in air temperature was noted at the end of the first decade of February - down to -3°C ... -4°C, and the minimum was -7.7°C. The temperature dropped at low air humidity and gusty winds. Under these conditions, the ratio F_v/F_m decreased in *Olea* genotypes by 25–30%, in *L. lucidum* by 15%, and in *Osmantus* species by 35% (*O. fragrans*) and 18% (*O.×fortunei*). Analysis of the leaf tissues water deficiency effect on frost resistance in *Olea* genotypes showed that its high level caused the decrease in their resistance to negative temperatures, in contrast to *Osmantus* and *Ligustrum* species [8,9]. The beginning of the cold period 2020-2021 characterized by high temperatures and was dry. In November, precipitation was only 5% of the norm. In December, the weather was mostly warm with cold waves during the passage of atmospheric fronts. The average daily air temperatures in January varied within +8.2…11.0°C, the maximum daytime temperature increased to +14.8°C. In the second decade of February, due to the penetration of Arctic air, the average daily temperatures dropped to negative values (-1 ... -3 ° C, on the soil surface the minimum temperature dropped to -8.0 ° C), and were often below the norm by 5 ... 7°C. The frosty period lasted 9 days. The studies of CFI parameters in Oleaceae plants with various frost resistance showed that during the cold period of 2020-2021, changes in the state of the photosynthetic apparatus were species-specific and were associated with weather conditions. In frost-resistant species *L. lucidum* and *O.×fortunei*, at the beginning of frost period, a decrease in the efficiency of light energy transfer in light-harvesting complexes, a decrease in F_v and F_m values were noted, while the photochemical activity of PS II changed insignificantly, by 10–15% on average. In *Olea europea* genotypes, on frosty days we noted an increase in initial fluorescence and a decrease in F_v/F_0 by 18-20%. That indicates the development of stress. At the end of the cold period, along with the chlorophyll a/b ratio increase, an increase of initial fluorescence (25–30%) was revealed in *Osmanthus* species. We suppose that it was associated with the restoration of the photosynthetic pigments fund. In *Olea europea* genotypes, the increase in F_0 did not exceed 10%. Such a picture may be related to the involvement of the newly formed chlorophylls in the processes of photosynthetic activity resumption [10].

To obtain more detailed information on the implementation of potential frost resistance in the studied species under specific weather conditions of the cold period and the effect of winter desiccation, we studied the dynamics of chlorophyll concentration in leaves, as well as the CFI parameters under the pressure of negative temperatures in combination with varied air humidity.

It was found that chlorophyll *a* concentration varied from 0.71 to 1.97 μg/g in terms of dry weight and chlorophyll *b* - 0.22-0.68 μg/g in terms of dry weight. The maximum content of chlorophylls *a* and *b* during the observation period was recorded in the leaves of *O. fragrans* and *O.×fortunei*.

According to literary sources, the chlorophyll *a*/*b* ratio may characterize potential photochemical activity and determine the degree of resistance in plants to unfavourable environmental factors. The normal ratio of chlorophyll *a* to chlorophyll *b* is 3:1 [11]. The presented studies demonstrated that in the beginning of the cold period 2020-2021 the chlorophyll *a/b* ratio varied within 3.16 – 3.89 (Fig.). In December, there was a decrease in this index, in all the studied genotypes by 5.5–16%. In the leaves of *O. fragrans*, it dropped below 3. In January, the coldest winter month, the chlorophyll *a/b* ratio decreased by additional 4–12%, in the leaves of *O. europea* cultivar 'Ascolano', *L. lucidum*, *O.×fortunei*. In *O. europea* cultivar 'Nikitskaya', this criterion did not change significantly, while in *O. fragrans* it increased to the maximum (3.47). In February, in all studied Oleaceae genotypes, a decrease in the chlorophyll *a/b* ratio to the minimum values were observed, the lowest values were recorded for species of the genus *Osmantus* (2.48).

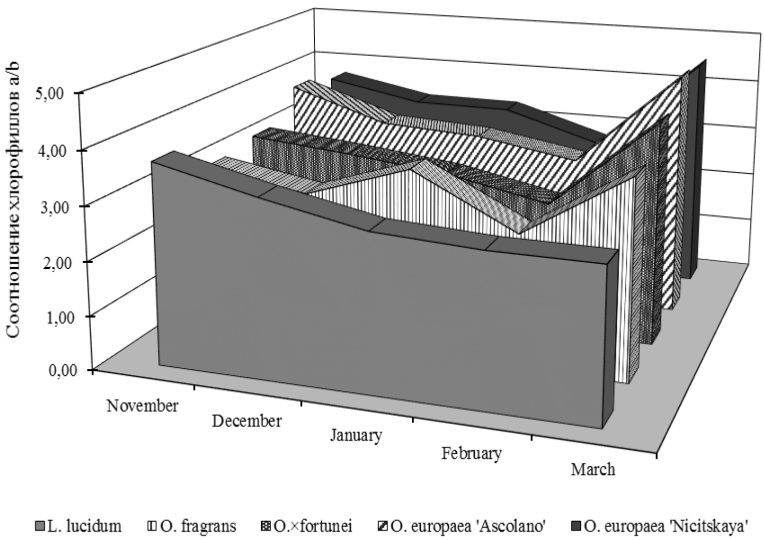

Fig. 1. Dynamics of the chlorophyll *a/b* ratio in the leaves of Oleaceae genotypes during the cold season 2020-2021.

This dynamics may be due to both the dry conditions throughout the cold period of 2020-2021 and the decrease in air temperature to negative values, accompanied by strong winds and low air humidity in February. At the end of the cold period, when the pressure of the stress factors decreased, there was a significant increase in the ratio of photosynthetic pigments in *Olea* cultivars and *Osmantus* species (by 33–40%). In *O. europea* cultivars 'Ascoliano', 'Nikitskaya' and *O.×fortunei*, the chlorophyll *a/b* ratio exceeded 4.2 that may indicate the restoration of a high level of potential photochemical activity [12]. At the same time, in the leaves of *L. lucidum*, this indicator remained at the minimum values of February (2.78) and, most likely, it was due to the lower adaptive capacity of this species.

Studies of the photosynthetic apparatus state in evergreen species of Oleaceae family, under the imitation of winter desiccation, showed that changes of the CFI parameters are species-specific. It has been found that in all the studied species, under the pressure of negative temperatures, the maximum and variable fluorescence decreased, and it was more pronounced at low relative air humidity. In *Olea* genotypes, a decrease in stationary and base fluorescence was observed (table). The cultivar 'Ascoliano' had irreversible disorders in PS II, that is evidenced by the absence of pronounced peaks on the CFI curve. In *Osmanthus* species, under the effect of various combinations of negative temperature and air humidity, diverse changes in the main CFI parameters were revealed. Thus, in a relatively frost-resistant species *O.* x *fortunei* at -8°C and 60% relative air humidity, slight changes in the CFI parameters were noted, and at low humidity (30%), the initial, stationary, maximum, and variable fluorescence decreased by an average of 20-25%.

These changes were not ultimate, since the vitality index (F_v/F_{st}) remained within the limits of the vitality norm. In the less resistant species *O. fragrans*, in variant 1, an increase in the values, which determines the level of energy expenditure during energy migration through the pigment matrix, was noted. At the same time, the value of fluorescence at the time of its temporary slowdown increased by 85%, which may be associated with disorders in the processes of reoxidation of Q_A - by Q_B [12].

Table. Changes in the chlorophyll fluorescence induction parameters in species of the Oleaceae family at various combinations of negative temperatures and air humidity.

CFI parameters / Variants	F_0	F_{pl}	F_m	F_{st}	F_v	F_v/F_{st}	F_v/F_m	F_v/F_0	$(F_{pl}-F_0)/F_v$
Olea europea 'Nikitskaya'									
Control	431±11	798±13	1442±29	431±7	1009	2.32	0.71	2.33	0.36
Variant 1	336±9	592±16	1120±31	352±9	784	2.21	0.72	2.32	0.34
Variant 2	719±9	863±17	1167±24	575±13	449	0.77	0.36	0.61	0.31
Osmanthus x *fortunei*									
Control	368±8	784±11	1344±31	352±6	976	2.77	0.73	2.65	0.43
Variant 1	352±12	752±10	1472±28	354±6	1120	3.16	0.76	3.18	0.36
Variant 2	288±6	544±12	1008±26	256±3	720	2.81	0.71	2.5	0.36
Ligustrum lucidum									
Control	296±8	576±14	1568±37	352±7	1272	3.61	0.81	4.30	0.22
Variant 1	294±11	496±16	1120±26	336±9	826	2.46	0.74	2.81	0.24
Variant 2	300±10	684±19	1344±29	329±5	1044	3.17	0.78	3.48	0.36
O. fragrans									
Control	224±4	560±14	1424±38	320±5	1200	3.75	0.84	5.36	0.28
Variant 1	496±13	1040±35	1776±42	448±11	1280	2.86	0.72	2.58	0.42
Variant 2	-	-	-	-					
Olea europea 'Ascoliano'									
Control	337±6	593±16	1231±29	433±10	895	2.06	0.72	2.61	0.28
Variant 1	303±3	497±12	1022±34	335±8	721	2.22	0.71	2.38	0.26
Variant 2	-	-	-	-					

Note. The significance of differences between the variants was assessed by Student's t-test at a the level of $P < 0.05$.

The effect of negative temperature and low air humidity caused the destruction of PS II structures.

Studies of the photosynthetic apparatus state in the species *L. lucidum*, which is quite high frost resistant, showed that more pronounced changes in the work of PS II were under the high air humidity: a significant decrease in the ratio of F_v/F_0 and F_v/F_{st} (by 35%) indicates a high stress level associated with an increase in non-photochemical energy losses. In contrast to variant 1, while simulating winter desiccation, a different picture was observed in this species: the $(F_{pl}-F_0)/F_v$ ratio increased by 63% that indicates an increase in the amount of unreduced Q_a in PS II reaction centers. At the same time, the viability index remained within the norm.

4 Conclusion

It has been revealed that changes in the functional state of the photosynthetic apparatus in some evergreen species of the Oleaceae family in winter seasons is species-specific and significantly depends on the weather conditions of a particular cold period. The pressure of negative temperatures along with air drought reduces the chlorophyll *a/b* ratio to a minimum in all the studied genotypes. With the diminution of the stress factors affect the concentration of chlorophylls was restored in *Olea* cultivars and *Osmantus* species, and in the leaves of *L. lucidum*, the ratio of photosynthetic pigments remained very low. Comparison of the obtained data with the CFI parameters lets us to suggest that the restoration of PSII functions in the species of *Olea* and *Osmantus* genera takes place due to the new chlorophylls formation after the end of stress pressure. In general, it can be concluded that the effect of negative temperatures in combination with low air humidity contributes to the development of deeper stress in the *Olea* and *Osmanthus* genotypes. However, *Olea* genotypes are more sensitive to winter drought. This is confirmed by the decrease in the F_v/F_{st} ratio to the lower limit of vitality in the frost-resistant cultivar 'Nikitskaya' and the irreversible inactivation of PSII in the cultivar 'Ascolyano', in the experiments simulating winter drought. It was found out that in the species *L. lucidum*, negative temperatures in combination with relatively high air humidity have a more significant effect on the state of the photosynthetic apparatus, which appears in an increase in energy expenditure during energy transfer through the pigment matrix and a decrease in the quantum yield of photosynthesis.

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