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SOME SPECIAL FEATURES OF PHOTOSYNTHETIC PROCESSES AND CHLOROPHYLL ACCUMULATION IN *OLEA EUROPEA* L. PLANTS ASSOCIATED WITH THEIR FROST TOLERANCE

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Abstract. In the last 90 years climate changes occurring on the Southern Coast of Crimea increase the risks of plant damage by negative temperatures during the cold period that is especially dangerous for deciduous evergreen non-native plant species. In order to reveal the special features of the photosynthetic apparatus functioning in *Olea europaea* subsp. *cuspidata* and *Olea europea* L. cultivars with different frost resistance, the dynamics of chlorophyll concentration in leaves was analyzed during cold periods on the Southern Coast of Crimea (SCC). A series of experiments to assess the stress level by changes in the photosynthetic processes were carried out under controlled conditions with an air temperature of -8°C and different relative humidity regimes - 60% and 30%. Chlorophyll concentration was determined spectrophotometrically. To analyze the state of the photosynthetic apparatus and identify the most sensitive to hydrothermal stress PS II links, the induced chlorophyll fluorescence (ICF) method was used. It was found out that low air humidity combined with negative temperatures caused disorder in the functioning of the oxygen-evolving complex in the frost-resistant cultivar 'Nikitskaya' and an increase in unreduced Qa in 'Correggiolo' cultivar, characterized by low resistance to negative temperatures. The pressure of negative temperatures together with high air humidity also caused a decrease in the maximum and variable fluorescence in *O. europaea* cultivars. However, there were no changes in the processes of plastoquinone reoxidation. In *Olea europaea* subsp. *cuspidata*, irreversible inactivation of PS II occurred under these conditions. It was revealed that during the cold period of 2019-2020, the total content of chlorophylls "a" and "b" in the leaves of *Olea europaea* subsp. *cuspidate* and *Olea europea* L. cultivars ranged from 0.91 to 2.54 mg/g dry weight. During the cold period in 2020-2021, significantly less photosynthetic pigments accumulated (0.52-1.9 mg/g), because of a long-drawn-out drought at the beginning. Frost-resistant cultivars were characterized by high chlorophyll content. Cultivars and subspecies with relatively low frost resistance showed a decrease in the chlorophyll amount after the frost onset. The potentially frost-resistant cultivar Nikitskaya is characterized by a more stable chlorophyll content during cold periods. The results obtained can be used to optimize *O. europaea* assortment in the regions where it is grown.
Keywords: *Olea europaea,* chlorophylls, frost resistance, photosynthesis

Climate changes significantly affect all areas of human activity. This problem is especially clear in the field of crop production in the Southern regions. Since it is here changes in the hydrothermal regime are associated with aridization, and accompanied by an increased probability of recurrent frosts and provocative thaws during the cold period. [1, 2]. Analysis of weather conditions on the southern coast of Crimea for over the past 90 years has shown that, despite the trend towards warming, the risks of plant damage by negative temperatures will increase. This is especially dangerous for deciduous evergreen non-native plant species [3, 4]. For *Olea europea*, the most dangerous are advections of cold air masses, often on the southern coast of Crimea, since we have previously found that a high level of water deficit in the leaves of *Olea europea* cultivars negatively affects their frost resistance [5].

Analysis of the existing scientific sources with the research results about the effect of negative temperatures on *Olea europea* plants, showed that most researchers do not take into account accompanying meteorological factors [6-8]. In this regard, studies of the complex effect of negative temperature and air humidity combinations on the functional state of the photosynthetic apparatus in *Olea europea*, as well as the dynamics of chlorophyll concentration during the cold period, allow us to reveal their contribution to the winter hardiness formation. Therefore, the goal of our studies was to identify the special features of chlorophyll accumulation in *Olea europea* cultivars and its subspecies *O. europaea* subsp. *cuspidata* during two cold periods and photosynthetic processes under various hydrothermal conditions.

MATERIALS AND METHODS

The objects of the investigations were the leaf apparatus of *Olea europea* cultivars 'Ascoliano', 'Coreggiolo', 'Nikitskaya' and its subspecies *O. europaea* subsp. *cuspidate* with various frost tolerance, which decreased in the line: 'Nikitskaya' - 'Coreggiolo' - 'Ascoliano' - *O. europaea* subsp. *cuspidata*. Critical temperatures are in the range of -8°C ... -14 ° C. [9]. The aim of the research was to identify the special features of chlorophylls "a" and "b" accumulation during the cold period on the southern coast of Crimea (SCC) and reveal the effect of air humidity on the photosynthesis processes under negative temperatures. Leaves selected from middle part of a one-year shoot were used for the research. The total chlorophyll amount was determined in the leaves during the cold periods (November-March) 2019-2020 and 2020-2021 by spectrophotometric method [10]. Experiments on the air humidity effect under pressure of negative temperatures were carried out on leafy annual shoots in the climatic camera (Binder MKF 115). Variant 1 – the effect of a temperature of -8°C for 6 h., at a relative humidity of 60%. Variant 2 – the effect of a temperature -8°C for 6 h., at a relative humidity of 30%. Leafy shoots maintained at a temperature of + 8 ° C ... + 10 °C for 12 h. were used as a control. The level of real water defecate in the leaves of cultivars and subspecies *O. europaea* was determined using the M.D. Kushnirenko method during the research period [11].

The state of the photosynthetic apparatus was assessed by the method of chlorophyll fluorescence induction using a fluorometer – "Floratest" [12]. The following parameters were analyzed: F_0 – basic fluorescence; Fm – maximum fluorescence; Fpl – fluorescence at the time of its temporary deceleration; Fs – stationary fluorescence level. On the basis of the measured parameters, we calculated the variable fluorescence (Fv), Fpl-F0)/Fv – the number of unreduced Qa in the reaction centers of PS II; Fv/F0 – the ratio of the speed constants in the reactions of photochemical and non-photochemical inactivation of excitation in PS II, as well as Fm/Fst – the fluorescence decay coefficient (viability index) and Fv/Fm – the efficiency of the light phase of photosynthesis [13]. The experiments were made in 3 replications. The obtained data were analyzed using MS Excel 2007 software. Significance in the differences between the variants was tested by Student's t-test at 0,05 level. The tables and graphs demonstrate the means and their standard errors.

RESULTS

According to the data by agrometeorological station “Nikitsky sad” the cold period of 2019-2020 was characterized by higher temperatures, compared to 2020-2021. The average temperature was 7,8°C and exceeded the climatic norm by 3,3°C. The cold period 2020-2021 was more humid. Precipitation was 70% of the climatic norm, while from November to March 2019-2020 it was only 28%.

However, by the beginning of the cold period 2020-2021, a high water deficiency (up to 15-18%) was noted in the leaves of the studied *Olea europea* cultivars and subspecies. It developed due to the long prior drought. In general, in 2020, precipitation was 60% below the norm and 109 mm less than in the previous year. Such conditions resulted in a decrease in the potential frost tolerance of the *O. europaea* cultivars and subspecies, most significantly in *O. europaea* subsp. *cuspidata* and 'Ascolano' cultivar. Analysis of the total chlorophylls "a" and "b" amount in olive leaves, during the cold periods of 2019-2020 and 2020-2021 on the southern coast of Crimea, demonstrated that in 2019-2020, chlorophyll concentration in the leaves of *O. europaea* cultivars and subspecies, on average, was 35-40% higher than that in the cold period of 2020-2021.

Changes in chlorophyll amount in the leaves of weakly tolerant cultivars 'Ascoliano' 'Koreggiolo' and *O. europaea* subsp. *cuspidata* in the cold period of 2019-2020 were the same (Tabl. 1). In November, the maximum concentrations of pigments were revealed, and at the end of the cold period their amount was minimal. The leaves of the frost-tolerant cultivar 'Nikitskaya' were characterized by the maximum content of chlorophyll in the coldest month, January. The dynamics of chlorophyll concentration during the cold period of 2020-2021 also had a number of differences. In the cultivars 'Ascoliano' and 'Koreggiolo', undulating changes in concentration were noted. In the leaves of *O. europaea* subsp. *cuspidata*, a 35% decrease in the pigments amount was noted in December. Cultivar 'Nikitskaya' was characterized by slight fluctuations in chlorophyll concentration.

Table 1.

Total chlorophyll amount in the olive leaves in the cold periods of 2019-2021

Cultivars and subspecies	Chlorophylls “a” and “b” amount, mg/g				
	November	December	January	February	March
2019-2020					
'Ascoliano'	2,54±0,12	1,99±0,09	1,82±0,08	2,11±0,09	1,09±0,05
O. europaea subsp. *cuspidata*	1,18±0,06	1,10±0,06	0,94±0,05	1,05±0,06	0,91±0,04
'Koreggiolo'	2,32±0,11	2,06±0,10	2,21±0,10	2,40±0,12	2,16±0,10
'Nikitskaya'	2,34±0,12	1,92±0,09	2,45±0,13	2,12±0,08	1,79±0,08
2020-2021					
'Ascoliano'	1,32±0,07	1,67±0,07	1,47±0,05	0,96±0,04	1,24±0,03
O. europaea subsp. *cuspidata*	0,83±0,04	0,52±0,02	0,81±0,03	0,77±0,02	0,78±0,02
'Koreggiolo'	1,78±0,08	1,46±0,05	1,90±0,09	1,67±0,08	1,37±0,03
'Nikitskaya'	1,21±0,06	1,21±0,04	1,16±0,03	1,34±0,05	1,22±0,04

In variant 1, a decrease in the base, maximum, stationary and variable fluorescence was noted in the cultivar 'Nikitskaya' by an average of 23-25% (Tabl. 2). The decay coefficient, the efficiency of the light phase of photosynthesis, the ratio of the speed constants in the reactions of photochemical and non-photochemical inactivation of excitation and the number of unreduced Q_a in the reaction centers of PS II didn`t change. In variant 2, significant changes in the functioning of the photosynthetic apparatus in 'Nikitskaya' cultivar were found out: an increase in basic and stationary fluorescence by 66% and 33%, respectively, and a decrease in the ratios F_v/F_m, F_v/F_0, F_v/F_{st}. The same changes were noted when studying the state of the photosynthetic apparatus in 'Ascoliano' cultivar. In *O. europaea* subsp. *cuspidata*, destruction in PS II was recorded even under air humidity of 60%. As for the cultivar 'Koreggiolo', a slightly different picture was observed under the conditions of this experiment. Changes in FI parameters in variant 1 were insignificant. Under the conditions of variant 2, the variable fluorescence significantly decreased and the number of unreduced Q_a in the PS II reaction centers increased.

DISCUSSION

Summarizing the obtained data, we can conclude that the effect of a temperature -8°C for 6 hours at air humidity of 60% has a less damaging effect compared to the same temperature regime, but at a humidity of 30% for all studied cultivars and subspecies of *O. europaea*. Presented data correspond to the previous studies, which demonstrated that, in *O. europaea* species, a high level of water deficit in winter negatively affects their frost tolerance, in contrast to other evergreen plants of Oleaceae family [14]. The revealed changes in FI parameters in the cultivar 'Nikitskaya' under conditions of relatively high air humidity indicate a decrease in the efficiency of excitation energy transfer from light-harvesting complexes to the PS II reaction center and an increase in the amount of chlorophyll involved in

Table 2.

Chlorophyll fluorescence induction (FI) parameters in O. europaea cultivars and subspecies at different relative air humidity and temperature -8°C

Parameter	F0	Fpl	Fm	Fst	Fv	Fv/Fst	Fv/Fm	Fv/F0	(Fpl-F0)/Fv
'Nikitskaya'									
Control	432±14	800±21	1440±27	432±9	1008	2,33	0,70	2,33	0,37
Variant 1	336±11	592±14	1120±29	352±12	784	2,22	0,70	2,33	0,33
Variant 2	720±17	864±18	1168±24	576±14	448	0,78	0,38	0,62	0,32
'Ascoliano'									
Control	336±9	592±13	1232±23	432±10	896	2,07	0,73	2,67	0,29
Variant 1	304±11	496±12	1024±25	336±8	720	2,21	0,70	2,39	0,27
Variant 2	-	-	-	-					
O. europaea subsp. *cuspidata*									
Control	192±11	336±8	656±16	224±9	464	2,07	0,71	2,42	0,31
Variant 1	208±14	288±9	496±13	224 ±6	288	1,85	0,58	1,38	0,28
Variant 2	-	-	-	-					
'Koreggiolo'									
Control	272±11	512±22	1488±24	352±12	1216	3,45	0,82	4,47	0,20
Variant 1	272±15	560±21	1248±28	320±10	976	3,05	0,78	3,59	0,30
Variant 2	192±6	496±19	1024±23	288±19	832	2.89	0,80	4,30	0,37

energy transport [15]. Relatively low amplitude of fluctuations in chlorophyll concentration provides this cultivar to maintain a “pool” of photosynthetic enzymes, and to a certain extent stabilize the work of PS II under unfavorable hydrothermal conditions of the cold period. Despite its high potential frost tolerance, this cultivar is sensitive to the complex pressure of low air humidity and negative temperatures. The beginning of irreversible disorders in such conditions is evidenced by a decrease in Fv/Fst below the vitality norm. This reaction is probably due to a decrease in the viscosity of thylakoid membranes and abnormalities in the functioning of the oxygen-evolving complex in PS II [16-18]. Irreversible inactivation of PS II in *O. europaea* subsp. *cuspidata* under the air humidity of 60% is due to the fact that the temperature of -8°C is critical for this cultivars and subspecies [5]. In addition, *O. europaea* subsp. *cuspidata* is characterized by the lowest amount of chlorophylls. During the periods of air temperature drop to negative values, this subspecies showed a decrease in chlorophyll amount that reduces the “safety margin” in PS II [19]. Of great interest are the results of photosynthesis processes studies in the weakly tolerant cultivar 'Koreggiolo', which did not show any critical changes in FI parameters, in all variants of the experiment. This is probably due to the cultivar characteristics and the presence of mechanisms that ensure the functioning of the photosynthetic apparatus under conditions of cold desiccation. It has been previously reported that some species potentially tolerant to strong stress pressure can`t resist to prolonged stress, or to the complex stress factors pressure [20].

CONCLUSION

It was found out that chlorophyll accumulation in annual leaves depends on specific weather conditions. The drier conditions in 2021 caused a decrease in chlorophylls amount in all studied cultivars and subspecies of *O. europaea*. Cultivars characterized by low frost tolerance and *O. europaea* subsp. *cuspidata* accumulate less chlorophylls. Analysis of *O. europaea* photosynthetic apparatus functioning under pressure of negative air temperatures and different air humidity (30% and 60%) demonstrated that abnormalities in PS II functioning are most intensive at low humidity. It has been revealed that olive cultivar 'Koreggiolo', in spite of its low potential frost tolerance, is quite resistant to conditions imitating winter drying. Presented data can be used to select olive cultivars with a high adaptive capacity to the conditions of the northern border of the cultural area. Further studies of a number of physiological and biochemical parameters in both *O. europaea* cultivars and other evergreen species of the Oleaceae family will reveal characteristics for an objective diagnosis of their tolerance to a complex of stress factors, as well as explore the aspects of adaptation strategies within the family.

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ОСОБЕННОСТИ ФОТОСИНТЕТИЧЕСКИХ ПРОЦЕССОВ И НАКОПЛЕНИЯ ХЛОРОФИЛЛОВ У OLEA EUROPEA L. В СВЯЗИ С ИХ МОРОЗОСТОЙКОСТЬЮ

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Аннотация. Изменения климата, происходящие на Южном берегу Крыма в последние 90 лет повышают риски повреждения растений отрицательными температурами в холодный период, что особенно опасно для лиственных вечнозеленых интродуцентов. С целью выявления особенностей работы фотосинтетического аппарата у сортов *Olea europea* L. и подвида *Olea europaea* subsp. *cuspidata,* с различной морозостойкостью был проведен анализ динамики концентрации хлорофиллов в листьях в течение холодных периодов на Южном берегу Крыма (ЮБК). В контролируемых условиях при действии температуры воздуха -8°С и различных режимах относительной влажности 60% и 30% была проведена серия экспериментов по оценке уровня стрессового состояния по изменениям в течении фотосинтетических процессов. Концентрацию хлорофиллов определяли спектрофотометрическим методом. Для анализа состояния фотосинтетического аппарата и выявления наиболее чувствительных к гидротермическому стрессу звеньев ФС II, использовали метод индуцированной флуоресценции хлорофилла (ИФХ). Установлено, что низкая влажность воздуха в сочетании с отрицательной температурой вызывает нарушения в работе кислородвыделяющего комплекса у морозостойкого сорта 'Никитская' и увеличение невостановленных Q_a у сорта 'Кореджиоло', характеризующегося низкой устойчивостью к отрицательным температурам. Действие отрицательной температуры в комплексе с высокой влажностью воздуха также стало причиной снижения максимальной и вариабельной флуоресценции у сортов *O. europaea*. Однако изменений в процессах реокисления пластохинонов выявлено не было. У подвида *Olea europaea* subsp. *cuspidata* в этих условиях, произошла необратимая инактивация ФС II. Выявлено, что в холодный период 2019-2020 гг. суммарное содержание хлорофиллов «а» и «b» в листьях сортов *Olea europea* L. и подвида *Olea europaea* subsp. *cuspidata* колебалось в пределах 0,91-2,54 мг/г в пересчете на сухой вес. В холодный период 2020-2021 гг. фотосинтетических пигментов накапливалось значительно меньше (0,52-1,9 мг/г), что связано с длительной засухой в его начале. Морозостойкие генотипы характеризовались большим содержанием хлорофилла. У сортов с относительно низкой морозостойкостью наблюдалось снижение количества хлорофиллов после наступления морозов. Потенциально морозостойкий сорт Никитская отличается более стабильным содержанием хлорофиллов в течение холодных периодов. Полученные результаты могут быть использованы для оптимизации сортимента *O. europaea* в регионах ее выращивания.

Ключевые слова: *Olea europaea,* морозоустойчивость, хлорофиллы, фотосинтез