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# ASSESSMENT OF PIGMENT CONTENT ON WILD GROWING PLANTS IN MOUSSALA PEAK

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***Abstract.*** *The highest peak on Rila Mountain is Moussala (2925 m a. s. l.). Its climate conditions depend on the geographic location, peculiarities of relief, and atmospheric circulation, and a specific microclimate is formed. In alpine regions, conditions become extremely variable with the increase in altitude. Plants that are growing in alpine conditions are exposed to the combined impact of environmental factors such as altitude, prolonged UV irradiation, low temperature, etc. This study aims to compare and assess whether the pigment content of wild-growing species at Moussala peak changes in the two following years. As plant material was used following species: Saxifraga cymosa Waldst & Kit (Saxifragaceae), Anthemis carpatica Waldst. & Kit. ex Willd. (Asteraceae), Geum repens (Rosaceae), Doronicum columnae Ten.(Asteraceae), Achillea clusiana L. (Asteraceae), Allium sibiricum L. (Liliaceae) and Festuca valida (R.Uechtr.) Pénzes (Poaceae). Plants were collected from Moussala Peak in July-August, in two successive growing seasons of 2020 and 2021. Data for the average daily value of "Erythemal UV irradiance" response (UVE) for the experimental site at Rila Mountain (2925 m a. s. l.) were measured with a UV sensor. Average daily values for July and August are calculated. Photosynthetic pigment content was applied as an endpoint. It was obtained that genotype response varies depending on the environmental conditions of the studied year. Data for UV irradiation at Moussala peak for a period of two following years 2020-2021 show insignificant change. Radiation conditions at Moussala peak show that both years differed in UVE response, but this small change of UV irradiation for one year is probably insignificant. The levels of total chlorophylls, chl. a, chl. b and total carotenoids for most of the studied alpine plants measured for 2021 were insignificantly higher in comparison with those measured for 2020. Only for S. cymosa were evaluated significantly higher pigment levels in 2021 than those in 2020. The chlorophyll a/b ratio was stable for all studied wild species growing at this altitude. The chlorophyll a/b ratio also varies depending on the studied genotype and different radiation conditions of the years studied. Our preliminary data indicate a change in pigment content depending on the different environmental conditions in the respective years and alpine plants examined. Data for radiation conditions at Moussala peak show that both years differed in UVE response, but this small change of UV irradiation for one year is probably insignificant. Changes in pigment content in some of the studied alpine genotypes propose different adaptive strategies to overcome the environmental stress at this altitude. Because alpine conditions at Moussala peak are related to the various impacts of extreme environmental factors on the pigment content of plants, further studies are needed to understand the mechanisms of interaction of factors and plant response in the long-term aspect of time.*

***Keywords:*** *alpine species, altitude, chlorophyll a, chlorophyll b, chlorophyll a/b ratio, Moussala peak, total chlorophylls, total carotenoids, UV irradiation*

## 1. INTRODUCTION

The highest peak of Rila Mountain and the Balkan Peninsula is Moussala peak (2925.4 m a. s. l.) (42°10′45″N, 23°35′07″E). This peak is far away from different types of anthropogenic pollution because of its location. On the other hand, its geographic location provides accessibility to the Mediterranean as well as Continental air masses [1]. Based on these facts, the area of Moussala peak is suitable for complex and comparative studies related to the combined impact of different environmental factors. It was found that at high altitudes atmospheric conditions change rapidly and that leads to successive dramatic fluctuations in the UV irradiation. A recent comparative study of solar UV irradiation at the high mountain stations of BEO-Moussala (BG) and NAO-Rozhen (BG) reported that in mountains, the accumulated UV dose is higher [2]. That observation is due to different factors such as higher transparency, the lower thickness of the atmospheric layers, dynamic optical effects ("cloud passing" and additional UV light reflections), and also the speed of the wind. Some correlation was found between the shape of clouds and increased UV irradiation. UV-A and UV-B irradiation increase by 15% on partially cloudy days than on the representative clear-sky days. UV-C irradiation is also detected at the Moussala peak [2]. At Moussala peak has located Basic Environmental Observatory (BEO)-Moussala (Institute of Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences) which is with coordinates 42°11′N and 23°35′ E. This observatory is one of the few high-altitude stations where different measurements of high-mountain environmental

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parameters (aerosol, background radiation, cosmic rays, etc.) are made [3], [4]. As a part of the highest mountain, Moussala peak is characterized by high-mountain conditions such as intensive UV irradiation, extreme climate conditions, quickly changing air, changing environmental parameters, etc. On the other hand, the location of this peak is interesting for many researchers because of its position at almost 3 km altitude which provides a lack of impacts, which could disturb the homogeneity of the collected data for a relatively long period of measurements [5]. Some authors found that atmosphere warming at peak Moussala follows the pattern of the whole world and this tendency is especially visible in recent years [5].

It is well known that in mountains plant diversity decreased rapidly with increasing altitude. Moussala peak and the surrounding area are characterized by alpine-type of vegetation. That type of vegetation includes a suite of azonal scree and rock vegetation, calcareous and acid grasslands and sedge heaths, snow beds, soligenous mires, and spring vegetation. The most important habitats are grasslands, located above the tree line and associated with late snow cover (over 210 days). The soil type is mountain-meadow alpine with pH values of approximately 4.5 [6]. In alpine conditions plants are exposed to the impact of more than one environmental factor. The wild-growing species at this altitude have developed adaptive strategies to cope under extreme conditions (poor soil, prolonged UV irradiation, lower temperature, short summer, etc.). Some of these strategies are related to adjusting the chlorophyll (Chl. *a*, Chl. *b*, Chl. (*a*+*b*), and Chl. *a/b*) and optimizing photosynthesis. Chlorophyll content plays a key role in the main vital plant process-photosynthesis. It was reported that chlorophylls are with greater sensitivity to changes in environmental conditions than carotenoids. Thus, leaf chlorophyll content could be a good indicator of environmental stress; changes: in temperature, humidity, air, and soil pollutant levels [7]. Another important plant pigments are carotenoids, which could be found in all photosynthetic organisms. They are masked by chlorophyll and have a minimal impact on plant color. Carotenoids are important for protecting chlorophyll from intensive sunlight. It was shown that in cases of environmental stress, chlorophyll content decreases more rapidly than carotenoid content [7]. The method for measurement of pigment content is quick and easy and for this reason, research on pigment content is becoming more and more common. The measurement of the chlorophyll content could be an important indicator of the plant's life processes, which affect plant growth, productivity, and the yield of biomass. Based on the fact that pigment content is a variable parameter that depends on many factors, we hypothesize that different plant species, growing at Moussala peak would have different photosynthetic pigment content for several consecutive years depending on the environmental conditions. To check our hypothesis photosynthetic pigment content (total chlorophylls (*a*+*b*), chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio, and total carotenoids) was used as an endpoint.

This study aims to compare and assess whether the pigment content of wild-growing species at Moussala peak changes in the two following years.

## 2. Material and methods

### *2.1. Plant material*

Seven wild-growing plant species were chosen as plant material: *Saxifraga cymosa* Waldst & Kit (Saxifragaceae), *Anthemis carpatica* Waldst. & Kit. ex Willd. (Asteraceae), *Geum repens* (Rosaceae), *Doronicum columnae* Ten. (Asteraceae), *Achillea clusiana* L. (Asteraceae), *Allium sibiricum* L. (Liliaceae) and *Festuca valida* (R.Uechtr.) Pénzes (Poaceae).

All plants were at the same phenological stage of development. Samples were performed on fully expanded leaves in mature plants at the flowering growth stage. Plant samples of all seven wild species were taken for research in July-August, in two consecutive growing seasons of 2020 and 2021. Three following collections of the species were made each year at the studied location.

Plant material for biochemical investigations was collected in moisture paper and after cooling at 4°C was taken to the laboratory. Then the plant material was stored at -77°C immediately after sampling to keep it fresh for further use.

Model plant *Hordeum vulgare* L. (Poaceae), grown under standard laboratory conditions at the Institute of Biodiversity and Ecosystem Research, Sofia was used as control. The barley seeds were presoaked (1h) in tap water, germinated (for 24 h at 24°C) and grown as hydroponics in a grown chamber for 14 days. The conditions during the growing stage were 12-h light/photoperiod/12-h dark, constant temperature (20–22°C) and constant watering. Mature plants were used further in the experimental procedures given below.

### *2.2. Experimental site*

Plants were collected from Moussala peak at an altitude of 2925 m a. s. l., near to BEO-Moussala for two successive growing seasons of 2020 and 2021. Coordinates of this experimental site are 42°10′45″N and 23°35′07″E. Typical for this site is mountain-meadow alpine soil type with approximately pH=4.5 [6].

### *2.3. Radiation conditions at the experimental site*

The available data for the average daily value of "Erythemal UV irradiance" response (UVE) irradiation for the experimental site at Rila Mountain (2925 m a. s. l.) is given for two following years 2020 and 2021. The results are presented as Wh/m$^2$. Average daily values for July and August were calculated from measurements and the collected database (N. Tyutyundzhiev). The UVE response is measured by the UV sensor system and defined by ISO/CIE 17166:2019. The UV sensor system is at BEO-

Moussala, located at Moussala peak, Rila Mountain, Bulgaria at an altitude of 2925 m a. s. l. and with coordinates 42◦11′N and 23◦35′ E.

UVE is typically made up of about 17% UVA and 83% UVB for a clear sky around solar noon. The UVA/UVB sensor can typically measure the daily total of UVE irradiance with an uncertainty of 5%.

### *2.4. Photosynthetic pigments*

The content of photosynthetic pigments (total chlorophylls, chlorophyll *a*, chlorophyll *b,* and total carotenoids) was spectrophotometrically determined in fresh leaves samples according to the method of Arnon (1949) [8] with some modifications. Pigment extraction was carried out with an 80% aqueous solution of acetone. Fresh leaf samples were homogenized with the solvent at room temperature (20-22°C). After homogenization, the plant extract was filtered. The time for extraction was about 3-10 min. depending on the species. Absorption was measured at 663 nm, 645 nm and 453 nm. The concentration of pigments was expressed as mg/g fresh weight. The chlorophyll *a/b* ratio was calculated.

### *2.5. Data statistical analysis*

Experiments were repeated at least three times. Data points in the figures are mean values. Error bars represent standard errors of mean values. Where no error bars are evident, the errors were equal to or less than the symbols. One-way ANOVA with Tukey multiple comparison tests (GraphPad Prism 6.04 software, San Diego, USA) was performed to assess differences among samples from the experimental site and differences among samples from different years.

## 3. RESULTS

### *3.1. Radiation conditions at the experimental site*

Our previous study showed a visible tendency to increase the intensity of UV radiation with height for a period of three years (2017-2019) [9]. It was suggested that the change in UV irradiation for one year is insignificant (about 1%). In our next study [10] was found that the increase in altitude, increases UV irradiation. The lowest values of UVA and UVB were detected at the lower site. The present data for UV irradiation at Moussala peak for a period of two following years 2020-2021 (for July and August) show an insignificant change (see Figure 1).

According to observations and measurements of N. Tyutyundzhiev dependence on altitude is logarithmic [10].

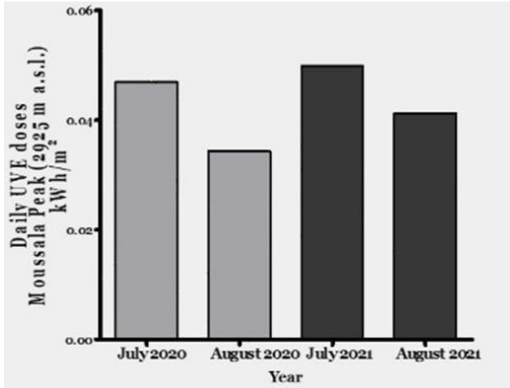


Figure 1. Daily UVE doses detected at the experimental site Moussala peak (2925 m a. s. l.) for July and August of the growing seasons 2020/2021 (kWh/m²).

### *3.2. Total chlorophyll (a+b) content*

A difference in genotype response depending on the environmental conditions of the studied year was found. The levels of total chlorophylls measured in 2021 for *A. carpatica, G. repens* and *F. valida* were insignificantly higher in comparison with those measured for 2020 (see Figure 2).

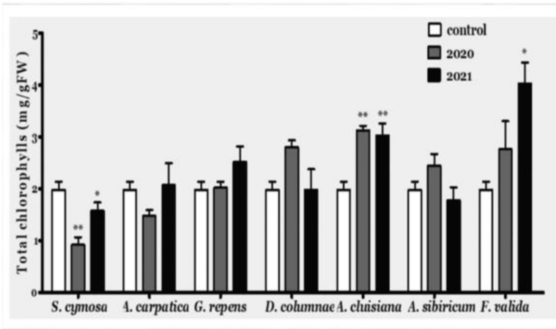


Figure 2. Total chlorophyll (*a*+*b*) content (mg/gFW). Statistically significant differences between: control vs. values for the year (*p<0.05, ** p<0.01); values for 2020 vs. 2021 (*p<0.05) for *S. cymosa*. *H. vulgare* L. is used as a control.

Total chlorophyll was with significantly higher levels only for *S. cymosa* in 2021 than those measured in 2020 (p<0.05). Lower and not statistically significant levels of total chlorophylls (p>0.05) were detected in 2021 for *D. columnae* and *A. sibiricum* than the ones detected in 2020.

### *3.3. Chlorophyll a and chlorophyll b content*

Higher but not statistically significant levels of chlorophyll *a* and chlorophyll *b* were obtained for *G. repens* in 2021 (p>0.05) compared with those measured in 2020 (see Figure 3).

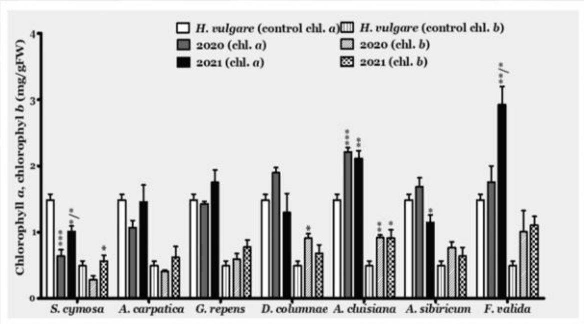


Figure 3. Chlorophyll *a* and chlorophyll *b* content (mg/gFW). Statistically significant differences between: control vs. values for year (*p<0.05, ** p<0.01, *** p<0.001 (for all species and before the slash); values for 2020 vs. 2021 (*p<0.05) for *S. cymosa*-chl. *a* (after the slash), chl. *b*; *A. sibiricum*-chl. *a*; *F. valida*-chl. *a* (after the slash). *H. vulgare* L. is used as a control.

Significantly higher chlorophyll *a* level for 2021 was obtained for *S. cymosa* and *F. valida* than those measured in 2020 (p<0.05). Chlorophyll *b* levels in 2021 for *S. cymosa* were also significantly higher than those in 2020 (p<0.05). Lower chlorophyll *a* content in 2021 was measured for *A. sibiricum* than the ones measured in 2020 (p<0.05).

### *3.4. Chlorophyll a/b ratio*

The chlorophyll a/b ratio was stable for all studied wild-growing species at this altitude (Table 1).

Table 1. Chlorophyll *a/b* ratio in wild genotypes growing at Moussala peak presented as Mean value ± SEM

| **Chlorophyll *a/b* ratio** | | |
|---|---|---|
| **Genotype** | **2020** | **2021** |
| **Control (*H. vulgare* L.)** | 3.141±0.290 | 3.141±0.290 |
| ***S. cymosa* Waldst&Kit** | 2.387 ± 0.229 | 1.951 ± 0.226 |
| ***A. carpatica* Waldst.&Kit. ex Willd** | 2.610 ± 0.279 | 2.408 ± 0.221 |
| ***G. repens* L.** | 2.504 ± 0.239 | 2.607 ± 0.276 |
| ***D. columnae* Ten.** | 2.110 ± 0.109 | 2.145 ± 0.355 |
| ***A. cluisiana* L.** | 2.420 ± 0.098 | 2.699 ± 0.315 |
| ***A. sibiricum* L.** | 2.237 ± 0.078 | 1.866 ± 0.215 |
| ***F. valida* (R.Uechtr.)Pénzes** | 2.002 ± 0.333 | 3.425 ± 0.696 |

Some variety in chlorophyll *a/b* ratio was also found depending on the genotype and different radiation conditions of the studied years.

### *3.5. Total carotenoid content*

Our data showed that the total carotenoid content for *S. cymosa, A. carpatica* and *G. repens* measured in 2021 was insignificantly higher in comparison with those measured in 2020 (p>0.05) (see Figure 4).

No significant difference was found between the levels of total carotenoids in 2020 and 2021 for all alpine species. The genotype with higher carotenoid content for both studied years is *F. valida* in comparison with other alpine genotypes.

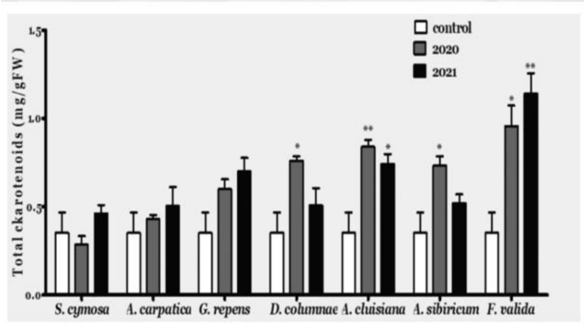


Figure 4. Total carotenoid content (mg/gFW). Statistically significant differences between: control vs. values for the year (*p<0.05, ** p<0.01). *H. vulgare* L. is used as a control.

## 4. Discussion

Photosynthetic pigments are important bioactive molecules in plants and their concentration varies depending on geographic region, season, and leaf condition. Plant pigment concentration could also vary depending on the species, and local environmental, bio-geological, and bio-geochemical factors [11]. As mentioned above, in mountains impact of the elevation is combined with increased UV intensity, rapidly changing air, quickly changing climate conditions, etc. Thus, chlorophyll content monitoring could be a good candidate for assessing the combined effects of environmental factors in high-mountain and alpine conditions on plants. Previous high mountain interdisciplinary studies in Rila Mountain are conducted for about 20 years [13]-[15]. In the present study, we obtained information about the UVE from BEO-Moussala with two-year monitoring of pigments concentration of alpine species. Changes in pigment content for this period could be used as a valuable indicator for plant adaptation to the constantly changing environment on Moussala peak. In our previous study, the observations [10] were made at two different altitudes in Rila Mountain (1500 m a. s. l. and 1782 m a. s. l.) for one year, with the top leaves of five herbaceous plants. We obtained that total chlorophyll content in *F. vesca, M. sylvatica, A. millefolium, E. angustifolium,* and *D. glomerata*, growing at both altitudes in Rila Mountain was lower or similar to that measured at control altitude (595 m a. s. l.). It is well known that in mountains, UV intensity increases with the increase in altitude. We found that genotype response, measured as total chlorophyll content varies depending on the species, and studied altitude [10]. The present study assessed the pigment content of seven wild-growing plant species (*S. cymosa, A. carpatica, G. repens, D. columnae, A. clusiana, A. sibiricum, F. valida*) at Moussala peak, Rila Mountain for a period of two consecutive years (2020-2021). Here we obtained that genotype response, measured as total chlorophyll content varies depending on the species and the studied year. This finding is in agreement with the studies of other authors [12], [13], [14], [15]. It was reported that the accumulation of chlorophyll (*a*+*b*) content is species-specific when eight phytomonitor species from Rila Mountain were examined [13]-[15]. We found that the levels of total chlorophylls in 2020 and 2021, for *S. cymosa, A. carpatica,* and *G. repens* were lower or similar to that of the control plant *H. vulgare* grown under standard laboratory conditions at a lower altitude. On the other hand, the levels of total chlorophylls in 2020 and 2021, for *A. clusiana* and *F. valida* were higher than the control ones. Even though, there's a difference between 2020 and 2021, revealed as total chlorophyll content these differences are insignificant. *S. cymosa* is the only genotype with significantly higher total chlorophyll levels in 2021 than those measured in 2020 ($p<0.05$). This observation is in accordance with another study [12]. Comparing the content of chlorophyll (*a+b*) in mountain melick plants (*Melica nutans* L.) growing in two forest habitats in the Zielonka Forest Landscape Park in the Greater Poland region, with different soil and moisture properties, it was found that total chlorophyll content in *M. nutans* in the first year of the study was significantly higher than in the second year.

One of the typical characteristics of alpine plants is reduced chlorophyll content. The chlorophyll content could be a variety-specific and species-specific characteristic [7], [10], [12]-[15] due to changes in altitude and high-mountain environmental conditions. Our data show that changes in chlorophyll *a* and chlorophyll *b* content could be due to: species-specific features, UV intensity of studied year and rapid decrease of chlorophyll *b* content than the chlorophyll *a* content. The levels of chlorophyll *a* and chlorophyll *b* for two studied years, for most of the alpine species, were lower or similar to the control. Statistically significant reduced levels of chlorophyll *a,* for two studied years, were obtained only for *S. cymosa*. On the other hand, for this genotype higher chlorophyll *a* content was obtained in 2021 than that in 2020. Opposite to this genotype, significantly higher levels of chlorophyll *a* than those of the control ones were measured for *A. clusiana* (for two studied years) and *F. valida* (for 2021). However, a significant difference between 2020 and 2021 was found only for genotype *F. valida*. Our data are in agreement with data of [10], [12]-[15]. Zielewicz et al., 2020 [12] obtained that the content of chlorophyll *a* in *M. nutans* in the first year of the study was higher than in the second year. In the present study, we observed significantly higher chlorophyll *b* levels in 2021, only for genotype *S. cymosa* than those in 2020. Higher chlorophyll *b* content than that of the control ones was measured for *A. clusiana*. For this genotype, no significant difference between the pigment levels for the studied years was found. However, chlorophyll *a* content was higher than that of chlorophyll *b* for all alpine species, for two studied years. Similar or lower data for chlorophyll *a* and chlorophyll *b* were obtained in our previous study [10], at both altitudes in five wild species, than those measured in plants at the control altitude. Variability among wild species exists in chlorophyll *a* and chlorophyll *b* contents. Species-specific changes of the chlorophyll *a* and chlorophyll *b* content in 69 mixed samples of eight phytomonitor species from 12 control sites, arranged in the 12 control populations in Rila Mountain during the three years (1994-1996) period were found [13]-[15]. Usually, chlorophyll *a* predominates chlorophyll *b* for most of the studied species. Changes in chlorophyll content characterized the resistance of plants and the extent of their adaptation to environmental stress factors. Based on the fact that UV irradiation increased with the altitude and insignificantly higher levels of UVE for 2021 than those measured for 2020 were noted at the Moussala peak, it could be suggested that species-specific changes in chlorophyll *a* and chlorophyll *b* content for seven alpine species are an adaptive strategy.

Another important indicator for plant photosynthetic activity and for plant status under specific conditions is the chlorophyll *a/b* ratio [13]-[15]. In higher plants, chlorophyll *a* has higher amounts of total chlorophyll, and thus, the ratio of chlorophyll *a* to *b* is about 3:1 [7] [11], [16]. Our data showed that, under specific alpine conditions at the Moussala peak for a two-year study period, values of the chlorophyll *a/b* ratio were in a close but normal range (3:1), for all wild-growing species. However, some variety in this ratio was found depending on the studied genotype and studied year. There's a slight difference in UVE values between 2020 and 2021 year. It could be suggested that an insignificant change in the environmental status of Moussala peak was observed for the 2020-2021 period. For the period of one year, a slight decrease in chlorophyll *a/b* ratio was calculated for *S. cymosa, A. carpatica* and *A. sibiricum*. In contrary for the same period of 2020-2021, a slight increase in chlorophyll ratio was calculated for *G. repens, A. clusiana, D. columnae* and *F. valida*. Our data are in accordance with data of [13]-[15]. Species-specific changes in the chlorophyll *a/b* ratio were reported for eight phytomonitor species from 12 control sites in Rila Mountain during the three-year research period. The values measured for chlorophyll *a/b* ratio were not high but variety was found depending on the genotype. This finding indicates that experimental sites are not influenced by a serious environmental stress factor, such as air pollution, that would provoke more significant changes in the pigment content of the phytomonitors and natural fund of Rila Mountain. The decrease of chlorophyll ratio for a long time period could be used as an early bioindication for an environmental factor, which disturbs pigment synthesis and photosynthesis in general [13]-[15]. It is reported that the slight value of the chlorophyll ratio could be a sensitive biomarker for pollution and environmental stress [17]. On the other hand, increase in the chlorophyll a/b ratio with the increase in environmental stresses is considered as a protective mechanism.

The other most common group of plant pigments, typical for all photosynthetic organisms, are carotenoids. They are secondary metabolites of plants, which as auxiliary pigments, transfer the absorbed energy to chlorophyll and also protect chlorophyll from excessive light intensity [7]. We found similar levels of total carotenoid content to those in the control plant, for *S. cymosa, A. carpatica,* and *G. repens* in two studied years. A lack of significant difference between the levels of total carotenoids in 2020 and 2021 for all alpine species could be an indication that there are no deviations in UVE levels at this altitude. The following higher-order concerning total carotenoid content was established for 2020: *A. sibiricum*>*D. columnae*>*A. clusiana*>*F. valida* and for 2021: *A. sibiricum*=*D. columnae*>*A. clusiana*>*F. valida*. Other authors have found that the carotenoid content in *M. nutans* was significantly differentiated by the meteorological conditions in the two subsequent years of the research [12]. Our data are opposite to data from our previous study where the levels of total carotenoids of five plant species, growing at 1500 m a.s.l and 1782 m a.s.l. were lower or similar to those at the control altitude. The present results are in accordance with data of [13]-[15]. The specific of the species in respect of carotenoid accumulation was demonstrated when 8 phytomonitor species from 12 different experimental sites in Rila mountain for a three-year period were studied. It was suggested that higher carotenoid content plays a protective role to chlorophylls, especially chlorophyll *a* [13]-[15]. The results obtained by us also supposed that higher carotenoid content for both studied years for genotype *F. valida* could be a protective strategy developed by the plant. Having in mind that our study is for a period of two years, observed changes in UVE at Moussala peak the pigment status don't show significant destructive deviations of the natural fund at this altitude. It could be supposed that the environmental status of Moussala peak for years 2020 and 2021 is insignificantly changed. Photosynthetic pigment content is a reliable indicator of plant response to different stress levels of humidity, precipitation, UV irradiation, temperature, etc. Thus, over a long time period, this content could be a sufficient indicator for environmental pollutant levels in the air, in the soil, and in the water used by plants and could be used for phytomonitoring of the environmental fund at Moussala. Due to the alpine conditions at the Moussala peak and their impact on the plant pigment content, further long-term studies are needed to understand the mechanisms of interaction of factors and plant response in the long-term aspect of time.

## 5. Conclusion

Data for radiation conditions at Moussala peak show that both years differed in UVE response, but this small change of UV irradiation for one year is probably insignificant. Having in mind the following results: the insignificant slight decrease of chlorophyll ratio calculated for *S. cymosa, A. carpatica* and *A. sibiricum* for 2021 at Moussala peak was insignificantly slight; a slight increase of chlorophyll ratio calculated for *G. repens, A. clusiana, D. columnae* and *F. valida* for 2021; short study period of one year; the stable chlorophyll ratio for the genotypes (3:1); and similar UVE values for 2020 and 2021, it could be suggested that the environmental conditions at the altitude of 2925 m a. s. l., for the period of 2020-2021 are not stressful enough for the studied alpine plants. Change in pigment content in some of the studied alpine genotypes proposes their different adaptive strategies to overcome the environmental stress at this altitude and could be used for two-year monitoring of the environmental status in Moussala peak.

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