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Comparative analysis of photosynthetic indicators in freesia hybrids on the Black sea coast of Krasnodar region

Oksana Belous[1,2]*, Kristina Klemeshova[1], Olga Pashchenko[1]

[1]*All-Russian Research Institute of Floriculture and Subtropical, Sochi, Russia*
[2] *Sochi institute of Design, Business and Law, Sochi, Russia*

**Corresponding author: oksana191962@mail.ru*

Abstract

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The paper discusses some features in *Freesia refracta* pigment system and possible use of this option as a plant diagnostic characteristic on the resistance in limiting environments. The study included freesia cultivars and hybrid forms selected in the Russian Research Institute of Horticulture and Subtropical Crops, which had different flowering terms. A detailed analysis was provided on any changes in the photosynthetic pigments content during the growing process. There are parameters of slow chlorophyll fluorescence induction (vitality index, ratio of photosynthetic activity and estimated coefficient of photosynthetic activity) in the leaves of different cultivars and hybrid forms. In order to determine the dependences between photosynthetic pigments content and parameters of slow chlorophyll fluorescence induction in freesia cultivars and hybrid forms pair correlation coefficient; it was calculated between these features among all hybrids and cultivars in general. The relationship recorded between optical indicators, and structural features of the pigment system will allow us to develop a method of rapid diagnosis of photosynthetic activity in freesia on the Black Sea coast of the Krasnodar region.

Keywords: freesia; photosynthetic apparatus; plastid pigments; chlorophyll fluorescence; correlation

Modern photosynthetic studies widely use the methods based on the measurement and analysis of slow chlorophyll fluorescence induction (SCFI). Great prospects in this field are determined by fluorescent indicators integral. This allows us to use fluorescence parameters, aiming not only to obtain information about the functioning plants photosynthetic apparatus, but also to study the way various factors effect on photosynthesis (Karapetyan, Bukhov 1986; Berezina, Gurova 2006; Shimko et al. 2009). Thanks to the informative value as well as speed and non-problematic measurements, chlorophyll fluorescence is used to solve various tasks, including the study of photosynthetic apparatus response to various biotic and abiotic factors, in particular, assessing the viability and complex sustainability to various stressors in general (Budagovskaya et al. 2004; 2014; Berezina, Gurova 2006).

Freesia culture is among the most promising in obtaining cut production on the Black Sea coast of Krasnodar region. With its rich colour, flower fragrance and elegant form, freesia culture is becoming increasingly popular in Russia. The possibility to obtain cut products throughout the year and especially during the winter and early spring period makes the cultivation highly profitable.

Typically, in the autumn-winter and spring period freesia is cultivated in greenhouses heated to maintain the optimum temperature (depending on the stage of plant development from 8–10 to 18–20°C). The region on the Black Sea coast of

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Krasnodar is among the few in Russia, where the work is carried out with the plants in unheated greenhouses during their vegetation. In winter and early spring, the temperatures may be from 0 to –5°C some days, and in the second half of April and May – rise to 25–30°C. Extreme growing conditions related to drops in temperature, negatively affect the physiological state of the plants themselves, and as a result, become a reason for changes in flowering terms and duration and poor quality of cut material (Mokhno, Pashchenko 2012; Klemeshova, Pashchenko 2014). Currently, the problems of freesia cultivars resistance in unheated greenhouses within some physiological parameters remain unexplored. Due to the fact that photosynthetic activity is an indicator of plant functional state, it was decided to study the work in the assimilation apparatus within these growing conditions, especially since the fluorescence method is often used as a proxy indicator in plant stability as a whole (Sorokina et al. 1985; Stasik, Grigoryuk 2000; Korneyev 2002; Abramchik et al. 2004).

MATERIAL AND METHODS

The materials for the research were freesia cultivars (*Freesia refracta* Klatt) and new hybrid forms selected in the Russian Research Institute of Horticulture and Subtropical Crops, having different flowering periods. In field conditions, these plants were grown by segments on an experimental plot based in the Institute.

Cv. 'Georgiy Pobedonosets' (control) – plant height – 0.96 m, 3 peduncles in a plant, 9–10 flowers in the inflorescence, very large flowers, flower height – 0.08 m, diameter – 0.07 m, red flower petals, single flower type. The cultivar is resistant in the culture.

Cv. 'Melange' – the resistance in the culture is very good. Plant height – 0.81 m, 4–5 stems, flower height – 0.07 m, flower diameter – 0.07 m, flower – light lilac, throat – light yellow, spots – yellow. There are from 11 to 12 flowers in the inflorescence.

Hybrid form I-108-1 – the flower stalk – 0.32 m, the inflorescence – 0.10 m, the main colour of the flower – white, throat – white and cream, spots – yellow, flower diameter – 0.06 m, flower height 0.07 m, from 7 to 9 flowers in the inflorescence.

Hybrid form M-R-5 – plant height – 0.50 m, 2–3 peduncles, 4–7 flowers in the inflorescence, large flowers, flower height – 0.06 m, diameter – 0.08 m, raspberry-pink flower petals with a yellow throat, double flower.

Hybrid form P-30-1 – plant height – 0.45 m, 3 peduncles in a plant, 7–9 flowers in the inflorescence, flowers are large, flower height 0.07 m, diameter 0.06 m, yellow, single flower petals.

Hybrid form P-34-1 – plant height 0.53 m, 3–4 peduncles in the same plant, 8–10 flowers in the inflorescence, large flowers, the height of the flower – 0.06 m in the middle, the diameter – 0.06 m, petals are raspberry-purple, single flower.

Hybrid form P-28-1 – peduncle height – 0.33 m, inflorescence length – 0.07 m, the main colour is blue and purple, the colour of the throat – white, without spot, flower diameter – 0.07 m, flower height – 0.07 m, 9–10 flowers in the inflorescence.

The features of photosynthetic apparatus work (PA) in freesia were studied in dynamics during the whole growing season. The pigments were extracted by acetone (100%) from mature leaves (weighed 170 mg), using the method by Shlyk (1971). The content of photosynthetic pigments was determined by absorption spectra, shot by spectrophotometer PE 5400 VI (Russia). The amount of pigments in the extracts was calculated according to the formulas proposed by Ziegler and Egle (Shlyk 1971; Krause, Wies 1991).

The functional state of leaves photosynthetic apparatus was evaluated by chlorophyll fluorescence parameters (device LPT-3C, Russia) (Budagovskaya et al. 2004; 2014).

The data were statistically processed by Dospekhov (1985) correlation analysis, using the software package Microsoft Excel. The table 1 and 2 shows the average values and errors of mean.

RESULTS AND DISCUSSION

The studies of pigment complex in freesia plant have identified some patterns in photosynthetic pigments, as well as pigment complex dynamics and its dependence upon a cultivar.

Seasonal changes in the content of chlorophyll and carotenoids ratio are typical for research objects. As far as the leaf matures, the number of chlorophyll *a* (1.655 mg/g), chlorophyll *b* (0.761 mg/g) as well as the amount of carotenoids (0.969 mg/g) contained in these leaves gradually increases and achieve its maximum in late February–March (Fig. 1).

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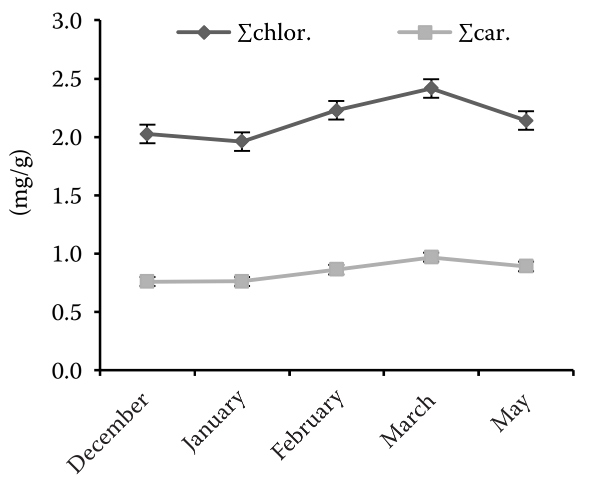

Fig. 1. Dynamics of photosynthetic pigments accumulation in freesia leaves is average for the culture
Σchlor. – sum of chlorophylls; Σcar. – sum of carotinoids

The patterns for the dynamics of photosynthetic pigments accumulation were identified, which are common to all the freesia cultivars, but resistant plants are characterized by less chlorophylls lability and larger carotenoids accumulation. In this process, it can clearly demonstrate varietal differences (Table 1).

The efficiency of the photosynthetic apparatus, as well as photosynthetic and biological plants productivity depends on the chlorophyll content. Plants photosynthetic efficiency is ultimately determined by the efficiency of light energy conversion in chloroplasts, as well as by the speed of photosynthetic metabolites formation and their use in the growth processes. The main photosynthetic pigment in freesia leaves is chlorophyll *a*, its amount ranges from 1.391 ± 0.21 mg/g (hybrid form P-28-1) to 1.620 ± 0.14 mg/g (hybrid form P-34-1). Hybrid forms P-28-1, P-30-1 and control cv. 'Georgiy Pobedonosets' differ from the rest of the samples by their lower chlorophyll content in the group (not significant difference). The value of chlorophyll *b* (whose content increased in more shade-tolerant plants) did not change significantly depending on a certain cultivar and was about 0.652 ± 0.02 mg/g. The highest amount of chlorophyll *b* was observed in the sample P-34-1 (0.694 ± 0.06). As a rule, the high content of chlorophyll *b* in the plants is preferred for photosynthetic activity, particularly in dense plantation.

Carotenoids are present in the membranes of all photosynthetic organisms, where they perform many important functions: aerial (optional pigments during solar energy absorption), protective (quenchers of triplet chlorophyll and singlet oxygen) and photoprotective (protect the reaction centre from powerful energy flows at high light intensities and stabilize the lipid phase of thylakoid membranes) (Alekhina et al. 2005). Elevated levels of carotenoids in plants are preferable in order to promote adaptive responses and reduce overall stress (Alekhina et al. 2005; Tsukanova 2007; Klemeshova, Belous 2013). As for the content of carotenoids between hybrid forms and earlier selected cultivars, there are no significant differences. Thus, the min. value was observed in cv. 'Georgiy Pobedonosets' (control) 0.796 ± 0.21 mg/g, the maximum was in hybrid form P-34-1 – 0.917 ±

Table 1. Characteristic of leaves pigment apparatus in freesia (different cultivars and hybrid forms)

Sample	C*a* (mg/g)	C*b* (mg/g)	Σchlor. (mg/g)	Σcar. (mg/g)	C*a*/C*b*	Σchlor./Σcar.
'Georgiy Pobedonosets'	1.44 ± 0.25	0.66 ± 0.15	2.11 ± 0.40	0.79 ± 0.21	2.22 ± 0.13	2.44 ± 0.20
'Melange'	1.51 ± 0.27	0.66 ± 0.10	2.17 ± 0.37	0.85 ± 0.16	2.28 ± 0.07	2.56 ± 0.07
I-108-1	1.58 ± 0.19	0.63 ± 0.17	2.21 ± 0.29	0.89 ± 0.12	2.26 ± 0.06	2.46 ± 0.11
M-R-5	1.50 ± 0.26	0.65 ± 0.13	2.15 ± 0.38	0.85 ± 0.14	2.31 ± 0.06	2.53 ± 0.08
P-30-1	1.46 ± 0.17	0.63 ± 0.12	2.09 ± 0.29	0.82 ± 0.13	2.35 ± 0.31	2.54 ± 0.06
P-34-1	1.62 ± 0.14	0.69 ± 0.06	2.31 ± 0.18	0.92 ± 0.08	2.34 ± 0.23	2.53 ± 0.09
P-28-1	1.39 ± 0.21	0.64 ± 0.09	2.03 ± 0/30	0.81 ± 0.12	2.16 ± 0.09	2.52 ± 0.11
$LSD_{0.05}$*	0.28	0.16	0.51	0.19	0.33	0.14

*least significant difference at the 95% significance level; C*a* – concentration of chlorophyll *a*, C*b* – concentration of chlorophyll *b*; Ca/Cb – ratio concentration of chlorophyll *a*/concentration of chlorophyll *b*; Σchlor. – sum of chlorophylls; Σcar. – sum of carotinoids; Σchlor./Σcar. – ratio sum of chlorophylls/sum of carotinoids

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0.076 mg/g. The difference of carotenoid content in the test samples compared with the control made up 1.5–11.2%.

Another important aspect in studying the pigment complex is, beside the content of certain pigments, also their ratio, since the relation between chlorophyll a and chlorophyll *b* (C*a*/C*b*) can show us shade tolerance. Under a severe lack of light, chlorophyll content is significantly lower than under normal lighting conditions. The ratio of C*a*/C*b* in light-demanding plants is the order of 3.9, while in shade-tolerant plants it is close to 2.3.

During the growing season, self-shadowing occurs in the bulk of freesia leaves, due to the cultivation conditions in greenhouses, which, however, is not optimal for this culture. According to C*a*/C*b* ratio, freesia relates to light-demanding plants and a self-shadowing is likely to have a stressful effect on leaves. The highest ratio of chlorophyll (C*a*/C*b*) was observed in hybrid forms P-30-1 (2.352 ± 0.31) and P-34-1 (2.342 ± 0.23), the smallest ratio – in hybrid form P-28-1 (2.158 ± 0.10), but the differences are not significant. It should be noted that the change in chlorophyll ratio (C*a*/C*b*) during the vegetation period is in antiphase with chlorophyll *b* (greater reduction in chlorophyll b leads to an increase in the C*a*/C*b* ratio).

The ratio of chlorophyll amount to carotenoids is a more informative feature, as it shows not only the degree plants are adapted to light, but also to adverse conditions. The lower is the ratio – the higher is plants resistance. According to the figures shown, the control cv. 'Georgiy Pobedonosets' (2.436 ± 0.20), and a hybrid form I-108-1 (2.463 ± 0.11) are the most notable, among the remaining samples this indicator was about 2.537 ± 0.01.

Slow chlorophyll fluorescence induction (SCFI) parameters, as well as the basic characteristics of the pigment apparatus in freesia leaves, are shown in Table 2.

Chlorophyll fluorescence quantitatively and qualitatively characterizes the photosynthetic apparatus in plants and its state, and slow chlorophyll fluorescence induction (SCFI) parameters are used as a stress indicator in plants. Sustainability Index (Fm/F_t) is calculated as the ratio of the fluores-

Table 2. The main characteristic of pigment apparatus and slow chlorophyll fluorescence induction (SCFI) parameters in freesia leaves (different cultivars and hybrid forms)

Sample	Σchlor. (mg/g)	Σcar.(mg/g)	Fm/F_t	Kf_T	Kf_n
Formation of flower spike					
'Georgiy Pobedonosets'	2.38 ± 0.09	0.91 ± 0.03	2.14 ± 0.19	0.52 ± 0.04	0.35 ± 0.03
'Melange'	2.05 ± 0.09	0.79 ± 0.05	2.25 ± 0.07	0.55 ± 0.02	0.38 ± 0.01
I-108-1	2.33 ± 0.04	0.91 ± 0.02	2.01 ± 0.07	0.50 ± 0.02	0.33 ± 0.01
M-R-5	2.17 ± 0.06	0.83 ± 0.02	2.64 ± 0.17	0.62 ± 0.02	0.43 ± 0.02
P-30-1	2.05 ± 0.24	0.79 ± 0.09	2.21 ± 0.11	0.54 ± 0.02	0.37 ± 0.02
P-34-1	2.35 ± 0.14	0.93 ± 0.06	1.90 ± 0.07	0.47 ± 0.02	0.33 ± 0.02
P-28-1	2.27 ± 0.02	0.87 ± 0.01	2,98 ± 0.20	0.66 ± 0.02	0.46 ± 0.02
$LSD_{0.05}$*	0.19	0.07	0.40	0.07	0.05
Flowering					
'Georgiy Pobedonosets'	2.53 ± 0.08	1.07 ± 0.03	3.77 ± 0.01	0.73 ± 0.01	0.52 ± 0.01
'Melange'	2.12 ± 0.14	0.84 ± 0.06	5.79 ± 0.48	0.82 ± 0.02	0.59 ± 0.01
I-108-1	2.54 ± 0.05	1.02 ± 0.02	3.19 ± 0.16	0.68 ± 0.02	0.47 ± 0.01
M-R-5	2.77 ± 0.20	1.08 ± 0.08	2.38 ± 0.08	0.58 ± 0.01	0.40 ± 0.01
P-30-1	2.26 ± 0.09	0.88 ± 0.03	3.48 ± 0.65	0.68 ± 0.05	0.47 ± 0.04
P-34-1	2.51 ± 0.06	1.02 ± 0.03	3.89 ± 0.24	0.74 ± 0.02	0.52 ± 0.02
P-28-1	2.19 ± 0,08	0.87 ± 0.03	4.14 ± 0.16	0.76 ± 0.01	0.53 ± 0.01
$LSD_{0.05}$*	0.19	0.08	0.98	0.07	0.05

*least significant difference at the 95% significance level; Σchlor. – sum of chlorophylls; Σcar. – sum of carotinoids; Fm – fluorescence maximum; F_t – stationary level of fluorescence; Fm/F_t – viability index; Kf_T – photosynthetic activity coefficient; Kf_n – coefficient of photosynthetic activity

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cence maximum to a steady level, it has no dimension or varietal and species specificity. Normally, the Fm/F_t has a value from 2 to 4, and is rarely more than 4 units. At the stage of flower spike formation the minimum viability index was observed in the hybrid form P-34-1 and it made up 1.900 ± 0.065. This indicator in hybrid forms P-28-1 and M-R-5 equals to 2.984 ± 0.203 and 2.644 ± 0.173, respectively; the difference is significant ($LSD_{0.05}$ = 0.40).

Photosynthetic activity coefficient (Kf_n) reflects the efficiency of light utilization in photosynthesis; it also has no dimension and does not depend on the species and varietal origin. The normal coefficient of photosynthetic activity is 0.6 and more, under various stressors it decreases in proportion to the weakening of photosynthetic function. The greatest value of this indicator was observed in the samples P-28-1 (0.461 ± 0.019) and M-R-5 (0.427 ± 0.016), the minimum in the sample P-34-1 (0.326 ± 0.015). The coefficient of photosynthetic activity in the remaining samples was about 0.359 ± 0.020; such low levels can be associated with stressful processes occurring in the chloroplasts, caused by the growing influence from adverse factors.

In practice, it is advisable to use other slow chlorophyll fluorescence induction (SCFI) parameters; thus the estimated coefficient of photosynthetic activity (Kf_T), logically identical to the parameter Kf_n, is determined by the estimated steady-state level of fluorescence for 30–60 s (by SCFI recipe). In practice, the higher is this indicator; the better is the functional state in plant organism. Among the researched samples, a minimum value for this parameter was observed in the hybrid form P-34-1 (0.471 ± 0.018) during the flower spike formation; as for the other cultivars and hybrid forms, it ranged from 0.500 to 0.553. Significantly different were the hybrid forms M-R-5 (0.616 ± 0.024) and P-28-1 (0.659 ± 0.021), for which the calculated ratio was significantly higher ($LSD_{0.05}$ = 0.07). However, this indicator is somewhat artificial and its use is justified in cases where it is necessary to carry out a large number of comparative measurements in a limited time.

During the transition to the reproductive development phase of SCFI the parameters changed a little bit. For instance, the most important index of viability was observed in cv. *Melange* (5.794 ± 0.481) and in the hybrid form P-28-1 (4.138 ± 0.155), while the lowest index was observed in the hybrid form M-R-5 (2.382 ± 0.079). The same dependance was recorded in both indicators of photosynthetic activity coefficient (Kf_ n), and in the data obtained from the calculated photosynthetic activity coefficient (Kf_T). The hybrid form M-R-5 had significantly lower values for all parameters in the flowering period, in contrast to the period when flower spike was formed. According to the considered cultivars and hybrid forms, it is possible to make a preliminary conclusion that the hybrid form P-28-1 is perspective, which is characterized (with its high SCFI parameters) by lower lability in green pigments and a large accumulation of carotenoids.

Table 3. Pair correlation coefficient (*r*) between the characteristics of the pigment apparatus and slow chlorophyll fluorescence induction (SCFI) parameters in freesia leaves (different cultivars and hybrid forms)

General correlation	Fm/F_t	Kf_T	Kf_n
Σchlor. (mg/g)	0.79	0.78	0.82
Σcar. (mg/g)	0.71	0.68	0.75
Ca/C*b*	–0.54	–0.54	–0.55

Ca/Cb – ratio concentration of chlorophyll *a*/concentration of chlorophyll *b*; Σchlor. – sum of chlorophylls; Σcar. – sum of carotinoids; Fm/F_t – viability index; Kf_T – photosynthetic activity coefficient; Kf_n – coefficient of photosynthetic activity

Based on the data, the correlation coefficients were counted between the main characteristics of the pigment system and slow chlorophyll fluorescence induction (SCFI) parameters in freesia leaves of different cultivars (Table 3).

In order to determine the dependence between the photosynthetic pigments content and slow chlorophyll fluorescence induction (SCFI) parameters in cultivars and hybrid forms, correlation coefficient was calculated between these features with all hybrids and cultivars in general. The dependence among the studied features in the whole culture was analysed and showed the close positive correlation between the total chlorophyll content (r = 0.82) and the amount of carotenoids (r = 0.75). It is worth noting that there is a negative dependance (r = –0.55) between chlorophyll ratio of and all slow chlorophyll fluorescence induction (SCFI) indicators.

CONCLUSION

Based on the preliminary data, conclusions can be drawn about the possibility to use the features

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in the pigment system (in freesia leaves) as a diagnostic feature in plants resistance to limiting environments. The relationship between the optical parameters used for rapid diagnosing of plants functional state based on drawing SCFI and structural features in the pigment system makes it possible to develop a rapid diagnostic of photosynthetic (assimilative) activity on the Black Sea coast of the Krasnodar region.

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