

CHANGES IN CHLOROPHYLL CONTENT AND FLUORESCENCE AND FRUIT YIELD CONTRIBUTING TRAITS IN DIFFERENT GENOTYPES OF STRAWBERRY (*Fragaria x ananassa* DUCH.)

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Abstract

Analysis of changes in chlorophyll fluorescence parameters in strawberry leaves was based on a field experiment performed in the years 2009–2010. Ten genotypes including 5 cultivars: ‘Kent’, ‘Teresa’, ‘Senga Sengana’, ‘Chandler’ and the breeding clone 1387 as well as their inbred progeny, were the object of the study. During the experiment the following indicators were evaluated: chlorophyll a and b content in fresh leaf mass as well as fluorescence parameters: minimum (F_0) and maximum fluorescence yield (F_m), photochemical efficiency of PS II (F_v/F_m), actual quantum yield of PSII photochemistry (Y), minimum (F_0') and maximum efficiency of fluorescence (F_m') in the light, coefficient of photochemical (qP) and non-photochemical (qN) fluorescence quenching. In this work, we also examined the effect of repeated inbreeding on strawberry fruit yield and yield components. The analysis of changes of these parameters showed that inbreeding caused a reaction in all tested cultivars. In all inbred progeny, chlorophyll a and b content decreased compared to the cultivars. Generally, the photochemical efficiency of photosystem II (F_v/F_m) and the parameter $\Delta F/F_m$ were not affected by strong inbreeding. In analyzing the values of the coefficients qP and qN , it has been observed that changes in their values depend on the sensitivity of the examined genotypes to self-pollination. The functioning of PS II is the most sensitive indicator of the effect of various factors on plants and is useful, among others, in breeding to select plants with a required genotype.

The yield – determining features such as: fruit yield per plant, weight of single fruit, number of fruit per plant and weight of leaves per plant in S_3 generation, were lower as compared with parental forms.

Key words: *Fragaria x ananassa* Duch., cultivars, photosynthetic activity, chlorophyll a and b, inbreeding depression

INTRODUCTION

Photosynthesis is a basic physiological process of plants with external and internal conditions. Measurement of chlorophyll fluorescence largely replaces the conventional measurements of the rate of photosynthesis and is a highly sensitive plant photosynthetic reaction attempt [1,2]. Chlorophyll fluorescence measurements are completely non-invasive, allowing photosynthesis to be studied *in vivo*. This method is particularly useful when the effects of different environmental factors [3,4,5,6], and biotic stress [7,8] on plants are studied.

Inbreeding generally increases homozygosity in plants and more homozygous genotypes may be susceptible to environmental harshness. In contrast, outcrossing usually increases or maintains heterozygosity that tends to produce genotypes better able to buffer themselves against environmental variability [9]. Inbreeding depression, the reduction in fitness of progeny derived from inbreeding relative to those derived from outcrossing, is invoked to explain the maintenance of the plant mating system [10].

Fragaria x ananassa ($2n = 8x = 56$), the cultivated strawberry, is a hybrid produced from a cross between the two New World octoploid strawberry species, *F. chiloensis* and *F. virginiana*, and is grown for its economically important fruit throughout the temperate regions of the world. Currently, there are over 100 breeding programmes worldwide, aiming to develop superior cultivars with improved flavour and texture, extended seasons of harvest, and a longer post-harvest shelf-life [11]. Experiments with strawberry have demonstrated significant and occasionally severe trait

mean depression for populations of inbred offspring constructed from matings among current-generation; fruit yields were depressed significantly even when rather modest rates of inbreeding were applied [12].

On the other hand, the level of genetic diversity in germplasm is a critical point of new cultivar creation, because inbreeding in cultivated plants leads to rapid loss in vigour, yield and fruit size [13].

We aimed to find a relationship between inbreeding depression and changes in the chlorophyll content and fluorescence. The presented research is innovative because of the lack of data in the literature on these subjects.

The aim of this study was to compare chosen photosynthetic parameters in the leaves of five strawberry cultivars and their inbred counterparts – third generation self (S_3). The study also evaluated the effect of inbreeding on yield, vigor and yield contributing characters in strawberry.

MATERIALS AND METHODS

The experiments testing the strawberry germplasm were conducted in the period 2009–2010 at the Felin Research Plantation of the University of Life Sciences in Lublin. Four strawberry cultivars: ‘Senga Sengana’ (‘Sieger’ x ‘Markee’), ‘Kent’ (‘Redgauntlet’ x ‘Tioga’) x ‘Raritan’), ‘Teresa’ (‘Redgauntlet S_1 ’ x ‘Senga Sengana’), ‘Chandler’ (‘Douglas’ x Cal 85.218–605), the breeding clone 1387 (‘Redgauntlet S_1 ’ x ‘Senga Sengana S_1 ’) x ‘Tioga S_1) and third generation inbreds derived from them were examined.

The first self-pollination was performed in May 2004. Before flowering time, inflorescences were covered with small bags made of cloth and left undisturbed for spontaneous selfing. 150 seedlings (S_1) derived from each of the cultivars (non-inbred progenies) were used in field trials on September 10, 2005. In May 2006, the second self-pollination was performed. Twenty seedlings randomly chosen from the first-generation self were self-pollinated to generate the second-generation self (S_2). Afterwards, 10 seedlings were retained from this population and used as parents to obtain the third-generation self (S_3). In June 2009, 10 seedlings derived from the same S_2 parental plants were planted on a separate experimental plot. Thus, the generation S_3 consisted of 100 plants of each cultivar (a total of 500 S_3 plants were examined).

Field measurements of the chlorophyll fluorescence parameters were performed using a fluorometer PAM-2000 by Walz GmbH, Germany. Chlorophyll fluorescence was measured on randomly selected, fully expanded triplet leaves that were in a similar physiological condition. The leaves were subjected to dark treatment for 20 min [14]. The measurements were

made in triplicate on the designated plants of each cultivar and inbred progeny on September 24, 2009, and September 20, 2010.

Over the study period, the following indices were recorded:

- chlorophyll a and b content in fresh leaf mass;
- F_0 – minimum efficiency of fluorescence;
- F_m – maximal fluorescence yield after dark adaptation;
- F_v/F_m – photochemical efficiency at PS II [15];
- Y – actual quantum yield of PSII photochemistry;
- F_0' – minimum efficiency of fluorescence in the light;
- F_m' – maximum efficiency of fluorescence in the light;
- qP – coefficient of photochemical fluorescence quenching;
- qN – coefficient of non-photochemical fluorescence quenching.

Chlorophyll a and b content in fresh leaf mass was determined by the spectrophotometric method described by Lichtenhaler and Wellburn [16].

During the research we also estimated: fruit yield per plant, fruit number per plant, average fruit weight, weight of leaves per plant. Data for growth and productivity traits were collected for individual plants (non-inbred cultivars and inbreeding populations) throughout the season following the plantation establishment. Weight of leaves per plant was obtained on July 20, after harvest. Yields and fruit number were recorded for each plant for 4 consecutive weeks starting from the first week of June. The weight of a single fruit was calculated by dividing weekly yields by corresponding fruit numbers. The results given are the average for two years of the research.

Variance analysis of the results obtained from the analyses and measurements was performed using Tukey’s test, defined in this work as $LSD_{0.05}$.

RESULTS

The functioning of photosystem II is the most sensitive indicator of various stress factors on plants. Changes in PS II activity can be determined quickly and non-invasively by measuring chlorophyll fluorescence (FC). The results obtained from FC induction measurements allow us to assess the progress and performance of photochemical reactions of light-phase photosynthesis leading to the formation of the so-called ‘*assimilation power*’ and its relationship to enzymatic reactions of photosynthesis. This in turn allows

us to determine the physiological state of plants growing in unfavorable environmental conditions.

In 2009 and 2010, the highest chlorophyll a content was found in the cultivar 'Chandler' ($1.32 \text{ mg} \times \text{g}^{-1}$), whereas the highest level of chlorophyll b was observed in 'Kent' and the clone 1387 ($0.40 \text{ mg} \times \text{g}^{-1}$). As shown in Table 1, there was a decrease in chlorophyll a (by an average of 10%) and chlorophyll b content (by 18%) in all inbred progeny. Due to this fact, the ratio of chlorophyll a/b content in inbred progeny averaged 3.66 as compared to the cultivars – 3.33.

Table 2 presents the changes in the chlorophyll fluorescence parameters in the leaves of the cultivars and their inbred progeny under field conditions. The minimum efficiency of fluorescence (F_0) showed a considerable variability, depending on clone, and ranged from 0.108 (1387 S_3) to 0.249 ('Teresa' S_3).

In our study, a decrease in maximum efficiency of fluorescence (F_m) was observed in all inbred progeny, on average by 19% compared to the cultivars, which indicates the photoinhibition changes in the strawberry leaves.

The maximum effect of photosystem II measured on dark-adapted strawberry leaves (F_v/F_m) was higher in the S_3 progeny by an average of 2% compared to the cultivars and the highest increase (by 13%) was observed in the 'Chandler' cultivar. The data presented in Table 3 indicate that, among the studied genotypes, inbred progenies of 'Chandler' were distinguished by

another reaction to selfing – there was an increase in fruit yield in S_3 and in number of fruits per plant.

The average value of the Y parameter in the S_3 progeny increased by 18% compared to the cultivars. In all offspring, with the exception of 'Kent' S_3 , an increase in this parameter was observed.

The results indicate that in all of the offspring (with the exception of the clone 1387 S_3) there was an increase in minimum efficiency of fluorescence in the light (F_0') which is expressed by an average increase of 17%. In 2009 and 2010 the clone 1387 and the progeny 'Teresa' S_3 had the highest maximum efficiency of fluorescence in the light (F_m' parameter = 0.410 and 0.361, respectively) of all 10 genotypes.

The highest and most favorable values of the photochemical fluorescence quenching coefficient (qP) were also found in 'Teresa' and therefore it is the least sensitive cultivar. In the present study, this strawberry cultivar was characterized by the most favorable ratio of the number of photons absorbed by PSII to the number of photons used by open centers in the reactions of photosynthesis. The value of the qN parameter decreased in all offspring, except for 'Chandler' S_3 plants.

As shown in Table 3, strong inbreeding caused a decrease in the value of all studied traits: fruit yield per plant decreased by 23%, weight of single fruit by 31%, number of fruit per plant by 48%, and weight of leaves per plant was reduced by as much as 67%.

Table 1
Chlorophyll content in strawberry leaves (average results for 2009–2010)

Cultivar(S_0)/Inbred progeny(S_3)	Chlorophyll content in $\text{mg} \times \text{g}^{-1}$ fresh mass		
	chlorophyll a	chlorophyll b	chlorophyll a/b
Senga Sengana (S_0)	1.182	0.341	3.47
1387 (S_0)	1.262	0.400	3.16
Teresa (S_0)	1.205	0.332	3.63
Kent (S_0)	1.232	0.401	3.07
Chandler (S_0)	1.320	0.398	3.32
Mean	1.240	0.374	3.33
LSD _{0.05}	0.044	0.032	0.12
Senga Sengana (S_3)	1.121	0.293	3.82
1387 (S_3)	1.082	0.299	3.62
Teresa (S_3)	1.093	0.301	3.63
Kent (S_3)	1.112	0.315	3.53
Chandler (S_3)	1.170	0.317	3.69
Mean	1.116	0.305	3.66
LSD _{0.05}	0.084	0.016	0.14

Table 2
Development of changes in chlorophyll fluorescence parameters in strawberry (average results for 2009–2010)

Cultivar(S ₀)/Inbred progeny(S ₃)	Chlorophyll fluorescence parameters							
	F ₀	F _m	F _v /F _m	Y	F _{o'}	F _{m'}	qP	qN
Senga Sengana (S ₀)	0.187	0.767	0.756	0.249	0.118	0.317	0.647	0.217
1387 (S ₀)	0.213	0.810	0.737	0.425	0.194	0.410	0.435	0.262
Teresa (S ₀)	0.198	0.867	0.772	0.406	0.173	0.343	0.672	0.221
Kent (S ₀)	0.197	0.823	0.761	0.462	0.120	0.339	0.572	0.190
Chandler (S ₀)	0.223	0.725	0.692	0.325	0.098	0.278	0.501	0.078
Mean	0.204	0.798	0.744	0.373	0.141	0.317	0.565	0.195
LSD _{0.05}	0.017	0.033	0.029	0.051	0.027	0.041	0.036	0.019
Senga Sengana (S ₃)	0.145	0.609	0.762	0.512	0.202	0.340	0.495	0.148
1387 (S ₃)	0.108	0.530	0.796	0.439	0.145	0.207	0.520	0.143
Teresa (S ₃)	0.249	0.818	0.696	0.560	0.202	0.361	0.534	0.100
Kent (S ₃)	0.159	0.641	0.752	0.354	0.153	0.207	0.465	0.177
Chandler (S ₃)	0.131	0.636	0.794	0.414	0.149	0.243	0.433	0.125
Mean	0.158	0.647	0.756	0.456	0.170	0.272	0.489	0.139
LSD _{0.05}	0.023	0.069	0.047	0.027	0.030	0.044	0.033	0.021

Table 3
Characteristics of strawberry cultivars and S₃ progeny in terms of yield and weight of leaves per plant (average results for 2009–2010)

Cultivar (S ₀)/ Inbred progeny (S ₃)	Fruit yield per plant (g)	Number of fruit per plant	Weight of fruit (g)	Weight of leaves per plant (g)
Senga Sengana (S ₀)	139.87	42.66	6.17	106.87
1387 (S ₀)	152.69	44.12	8.89	133.33
Teresa (S ₀)	197.20	51.30	9.02	172.50
Kent (S ₀)	128.17	46.53	8.07	75.00
Chandler (S ₀)	117.00	33.50	5.12	88.33
Mean	146.99	43.62	7.45	115.21
LSD _{0.05}	35.76	7.75	2.33	55.98
Senga Sengana (S ₃)	72.32	14.23	5.07	34.01
1387 (S ₃)	127.80	20.11	4.87	33.56
Teresa (S ₃)	104.00	18.41	5.88	41.71
Kent (S ₃)	118.44	24.53	4.71	38.24
Chandler (S ₃)	139.46	34.34	4.53	36.02
Mean	113.68	22.85	5.17	37.87
LSD _{0.05}	18.29	8.69	2.14	5.64

DISCUSSION

The genetic characteristics of the tested cultivars most differentiated the photosynthetic performance indicators in strawberry plants (Tables 1 and 2). Michałek and Sawicka [17] reported that the content of chlorophyll a and b in fresh mass of potato leaves depended on the cultivar and growth stage of

plants. The highest chlorophyll a content was found at full bloom, while that of chlorophyll b during emergence.

The F_v/F_m ratio in control plants is usually 0.80–0.83 and is a measure of potential quantum yield of photosystem PS II [18]. In the present work, the average values of potential quantum efficiency PS II (the parameter F_v/F_m) were similar in the leaves of inbred

and non-inbred plants. Similarly, R a z a v i et al. [6] demonstrated in strawberry that maximum efficiency of photosystem II was not affected by drought stress.

As a result of using the fluorescence method, a decrease in the efficiency of photosynthesis after stress caused by aphids feeding was found in the leaves of all the tested hazel cultivars [19,20,8].

A study by C i c h o c k a et al. [21] showed a slower rate of photosynthesis after a few weeks of preying by various species of aphids on all the studied host plants. A strong reaction of the photosystem to preying aphids was especially well visible in different species of trees and shrubs. For example, photosynthesis in hawthorn leaves injured by *Dysaphis crataegi* decreased 4–5 times as compared to the control [22].

In certain conditions, however, plants – due to induced defence – tolerate the presence of pests. The authors explain this phenomenon by growth stimulation, an increase in photosynthetic rate, a change in the distribution of assimilative substances or fast necrotization of tissues. In this way, they are capable of compensating the losses, which balances the negative effect of some biotic stress, thanks to which plants remain in a good condition and they yield well [23].

A study by G a n t n e r and M i c h a ł e k [8] showed that the ability to convert PAR photon energy to chemical energy in light conditions decreased in the leaves of all the tested hazel cultivars. The $\Delta F/F_m$ parameter was reduced on average by 27% after aphid feeding in comparison with the control.

On the other hand, poor infection of bean, strawberry, chrysanthemum and cucumber plants by red spiders caused an increased rate of photosynthesis, which contributed to increased metabolism and induction of a defensive reaction, leading as a consequence to losses and even growth stimulation and improved yielding [24].

The results from our study confirmed the data found in the literature concerning the great variability of non-photochemical chlorophyll fluorescence quenching (qN). According to S a w i c k a and M i c h a ł e k [25], this coefficient in sunflower plants was the least stable and ranged from 0.092 to 0.356, depending on variety. K r e b s et al. [26] also considered that indicator as not very stable.

In the study by R a z a v i et al. [6], the effect of water deficit on chlorophyll fluorescence, sugar content, and growth parameters of strawberry was investigated. A gradual reduction of photochemical quenching and quantum efficiency was observed under drought stress in strawberry cv. ‘Elsanta’, while non-photochemical quenching increased.

Inbreeding also caused changes in terms of vegetative and generative traits in strawberry plants. In this experiment, inbreeding depression resulted in si-

gnificant reductions in fruit yield per plant (by 22.7%), weight of single fruit (by 30.6%), number of fruit per plant (by 47.6%), and average weight of leaves per plant (by 67.1%).

In domestic strawberries, fruit yield is among the traits that are most affected by inbreeding and can be reduced by as much as 80% after two generations of self-fertilization [12]. Similarly, M e l v i l l e et al. [27] reported that inbred lines showed smaller plant size, stems and leaves, fewer crowns as well as a decrease in yield. A study by R h o et al. [28] also showed a decline in plant height and leaf area, with 9–31% decreases in the inbred lines in comparison with the original cultivars. In addition, fruit size and yield were reduced, whereas fruit quality was lower in terms of commercial value compared with the original cultivar.

B e l l u s c i et al. [29] indicates that the magnitude of trait mean depression depends on the rate at which homozygosity accumulates and the strength of selection pressure counteracting this depression. Additionally, it is supposed that reciprocal recurrent selection would give more viable and homogeneous inbred lines.

The magnitude of mean depression observed for the inbred progeny in this study is consistent with the results obtained by Ż u r a w i c z [30], where a strong inbreeding cultivar, ‘Dukat’, caused a very strong reduction in yield compared to the families obtained through sib-crossing and mating of non-related parents.

In the opinion of J a n s e n et al. [31] as well as of M i c h a ł e k and S a w i c k a [17], each cultivar produces assimilates in an individual way, as evidenced by different values of particular parameters of chlorophyll fluorescence obtained in the reported studies. D e m m i n g and B j ó r k m a n [18], M i c h a ł e k [32] as well as M i c h a ł e k and S a w i c k a [33] also indicate that chlorophyll fluorescence is a measure of the efficiency of photosynthesis and that its effectiveness may depend on the characteristics of the genotype.

In conclusion, our results demonstrate that inbreeding in strawberry induces changes in photosynthetic parameters, yield and plant vigour.

CONCLUSIONS

1. The presented research shows that strawberry cultivars differ in their sensitivity to inbreeding as demonstrated by significant differences in photosynthetic efficiency, generative traits and vigour of plants.
2. Strong inbreeding caused a decrease in chlorophyll a and b content in all offspring.
3. The following characters: fruit yield per plant, weight of single fruit, number of fruit per plant and weight of leaves per plant, were lower in the S₃ generation as compared with parental forms.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: design of the experiments: WM, EK; performance of the experiments: WM, EK; analysis of the experimental data: EK, WM; writing of the paper: EK, WM.

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Zmiany we fluorescencji chlorofilu i cechach plonotwórczych u różnych genotypów truskawki (*Fragaria x ananassa* Duch.)

Streszczenie

Badania dotyczące aktywności fotosyntetycznej liści truskawki oparto na wynikach doświadczeń polowych przeprowadzonych w latach 2009–2010. Badania objęto 5 odmian: ‘Kent’, ‘Teresa’, ‘Senga Sengana’, ‘Chandler’ i klon hodowlany 1387 oraz uzyskane z nich potomstwo wsobne. Podczas doświadczenia określono zawartość chlorofilu a i b w świeżej masie liści oraz parametry fluorescencji: minimalną (F_0) i maksymalną (F_m) fluorescencję, maksymalną sprawność fotosystemu PS II (F_v/F_m), wskaźnik $\Delta F/F_m'$ określający całkowity zysk kwantowy fotosyntezy, minimalną (F_0') i maksymalną (F_m') fluorescencję na świetle oraz współczynnik fotochemicznego (qP) i niefotochemicznego (qN) wygaszania fluorescencji. W pracy badano również wpływ chowu wsobnego na plon owoców i cechy plonotwórcze u truskawki. Z analizy danych wynika, że chów wsobny spowodował zmiany w wartościach powyższych parametrów u badanych odmian. U wszystkich potomstw wsobnych nastąpił spadek zawartości chlorofilu a i b w stosunku do odmian wyjściowych. Generalnie, silny chów wsobny nie zakłócał maksymalnej wydajności fotosystemu II (F_v/F_m) oraz rzeczywistej wydajności fotochemicznej związanej z efektywnością transportu elektronów ($\Delta F/F_m'$). Uzyskane rezultaty wykazały również, że wartości parametrów qP i qN są uzależnione od reakcji poszczególnych genotypów na samozapylenie.

W pokoleniu S₃ zaobserwowano spadek wartości następujących cech: plonu owoców z rośliny, liczby owoców z rośliny, średniej masy owocu i masy liści z rośliny w stosunku do form rodzicielskich.

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