THE VARIATION OF THE ASSIMILATING PIGMENTS CONTENT IN *GARDENIA JASMINOIDES* (Ellis) H.Br. CALLUS UNDER ACTION OF SPERMIDINE EXOGENOUS ADDED

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This study evaluated the quantity of the assimilating pigments of some types of *Gardenia jasminoides* calli under the action of Spd exogenous added. This was analyzed in comparison with leaves of the *in vitro* regenerated plants. The results have demonstrated that Spd 0.1 mM has determined a decrease in the quantity of the chlorophylls in the calli and increase of the carotenoid pigments. The diminishing of the total chlorophyll/carotenoid ratio on the primary calli confirms the apport of exogenous Spd take quantitative diminishing of the assimilating pigments as to the quantitative growth of the carotenoids.

Key words: Spd = spermidine, s.p. = fresh substance, *Gardenia jasminoides* (Ellis) H.Br.

INTRODUCTION

The plant morphogenesis process is complex and specific for each species. It is controlled directly by the endogenous factor (physiological age, and genetical factors) and by exogenous ones, like: temperature, light, different growth substances added to the culture medium. The culture age is a very important factor with potential influence on genetical expression of callus cells. The interaction of these factors has determined different responses regarding the assimilating pigments synthesis at the different calli levels in comparison with the assimilating pigments synthesis in the leaves of *Gardenia jasminoides* (Ellis) H.Br. Long-term callus and the primary callus have an appropriate morphology from some points of view but their growth rate is different (2).Dumitrescu (5) have been noticed in *Nicotiana tabacum* L. (cv. Xanthi) callus culture an important difference in assimilating pigment content under action of exogenous spermidine.

In the present study, some biochemical parameters have been evaluated for the purpose of understanding the differentiated response of calli of this species in comparison to the action of polyamines. There has been determined the quantity of the assimilating pigments, in some type of calli as primary callus under the treatment with spermidine and long term-callus in comparison to leaf of the *in vitro* regenerated plants.

MATERIAL AND METHODS

The quantitative evaluation of the assimilating pigments of *Gardenia jasminoides* (Ellis) H. Br. was realized in the long-term callus obtained on the standard culture medium MS(1962) modified by adding AIA 0.1 mg/l, BA 1 mg/l, agar 8 g/l, at the pH of 5.8 and the in primary callus obtained on the MS(1962) medium, modified and supplemented with 0.1 mM Spd. Primary callus obtained in the MS(1962) medium, without Spd was used as control. The quantities of the assimilating pigments of these types of calli, have been compared with of the chlorophyll recorded in the leaves.

The Petri plates with calli have been kept in tissue culture growing room with the following conditions: light intensity = 3 000 lucs, a photoperiod of 16 hours light and 8 hours dark daily and an average temperature of 26° C ± 2° C. The extraction of the assimilating pigments has been realized in 85 % acetone, according to Strain and Svec (12). The readings were done using Specool. For assimilating pigments calculation L.P. Vernon's formulas have been used.

RESULTS AND DISCUSSIONS

Chlorophyll *a* (Table 1, Figs. 1, 2)

In all of the three types of calli the quantity of the *a* chlorophyll- active pigment, is much diminished in comparison to the quantity of the *a* chlorophyll extracted from the leaves of the plant (1.51 mg/1 g s.p.). At the primary callus obtained on the MS(1962) culture medium, modified and added with Spd 0,1 mM, there has been remarked diminution of *a* chlorophyll (0.011 mg/1g s.p.), in comparison to the *a* chlorophyll extracted from the long-term callus (0.023 mg/1g s.p.) obtained on the MS(1962) culture medium without Spd. A considerable growth of the *a* chlorophyll (0,134 mg/1g s.p.) has taken place in the primary calli obtained in the MS(1962) without Spd.

Chlorophyll *b* (Table 1, Figs. 1, 2)

Chlorophyll *b,* considered an accessory pigment, having a role in the attraction of the light energy, has high quantitative values in comparison with the *a* chlorophyll from the same type of calli. In the primary callus obtained in the MS(1962) culture medium added to Spd 0,1 mM, the quantity of the *b* chlorophyll (0.018 mg/1g s.p.) is diminished in analogy to the one extracted from the long-term callus obtained on the standard culture medium MS(1962), without Spd (0.037 mg/1g s.p.), also with the *b* chlorophyll in the primary callus without Spd (0.24 mg/1g s.p.). The value of the *b* chlorophyll content in the leaf (1.03mg/1g s.p.) is superior to the one obtained in the callus and diminished in comparison to the value of the *a* chlorophyll content in the leaf.

The total chlorophyll (Table 1, Figs. 1, 2)

The quantity of the chlorophyll at the primary callus obtained on the culture medium MS(1962) modified and added with Spd 0,1 mM (0.30 mg/1g s.p.) representing half of the total quantity of the chlorophyll present in the long-term callus (0.060 mg/1g s.p.). The highest values of the total chlorophyll have been registered in the primary calli without Spd (0.38 mg/1g s.p.), also in the leaves (2.56 mg/1g s.p.).

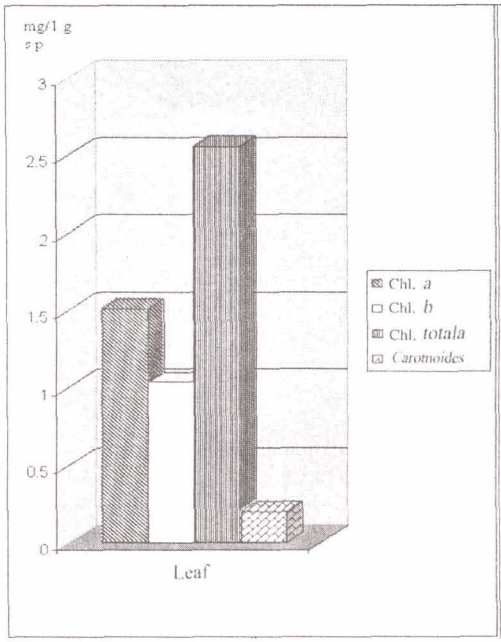

Fig. 1 – The content in assimilating pigment of the leaf of Gardenia jasminoides (Ellis) H. Br.

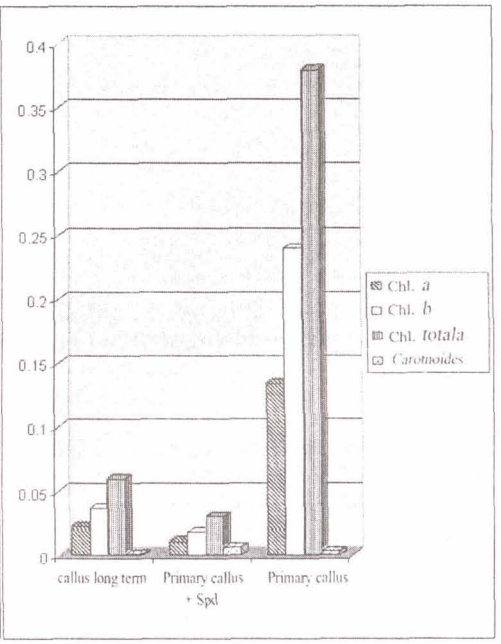

Fig. 2 – The content in assimilating pigments of the calli of gardenia jasminoides (Ellis) H. Br.

The *a/b chlorophyll ratio* (Table 1, Fig. 3)

Although very close to the value of the studies calli, having values between 0.62 in the long-term callus and 0.55 in the primary callus obtained in the culture medium without Spd, the chlorophyll ratio *a/b* is diminished in comparison to the chlorophyll ratio *a/b* in the leaf (1.46). Vasil I. K. *et al.* (13) have estimated that the diminishing of the chlorophyll ratio *a/b* indicates a degrading of the synthesis of the active pigment. In all the calli take into study, there has taken place a diminishing of the values of *a* chlorophyll, in comparison with values of *b* chlorophyll.

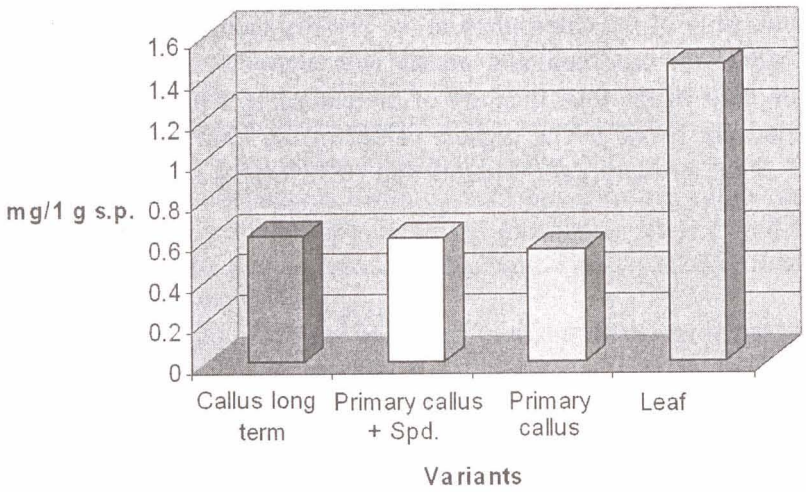

Fig. 3 – The chl a/b ratio.

The carotenoid pigments. (Table 1, Figs. 1, 2)

Table 1

The content of assimilating pigments of *Gardenia jasminoides* (Ellis) H.Br. in variety types of calli and leaves. mg/1g s.p.

Variants	*"a"* chloroph.	*"b"* chloroph.	*total chloroph.*	*a/b* ratio	Carotenoid.	chl. *tot*/*carot* ratio
Long-term callus	0.023	0.037	0.060	0.62	0.001	60
Primary callus MS(1962)+ Spd 0.1mM	0.011	0.018	0.030	0.61	0.006	5
Primary callus	0.134	0.24	0.38	0.55	0.003	12.6
Leaf	1.51	1.03	2.56	1.46	0.2	12.8

In contradiction with the *in vitro* regenerated plants in which the values of the carotenoid pigments represent 0.2 mg/1g s.p., in the callus obtained *in vitro* a very small amount of carotenoid pigment has been decelated. The highest value, (0.006 mg/1g s.p.) has been registered on the primary calli obtained in MS(1962)

culture medium supplemented with Spd 0.1 mM. In long-term callus there has been registered 0.001 mg/1g s.p., and in the primary callus obtained in MS(1962) without Spd 0.003 mg/1g s.p.

The obtained results have demonstrated that Spd has determined a decrease in the quantity of the chlorophyll pigments in the calli and an increase of the carotenoid pigments.

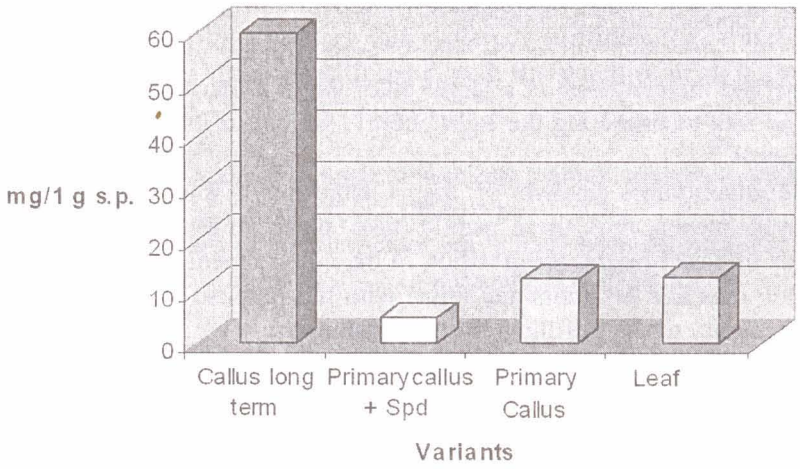

Fig. 4 – Total chlorophyll/carotenoid pigments ratio.

Total chlorophyll/carotenoid pigments ratio (Table 1, Fig. 4).

The total chlorophyll/carotenoid pigments ratio present high values in the calli obtained on the culture medium without spermidine, which demonstrates the prevailing of the assimilating pigments. On the primary calli obtained in MS(1962) culture medium added with Spd 0.1 mM, the total chlorophyll/carotenoids ratio is small (5). The demising of this ratio shows a carotenoid growth as a sequence of the modifying of the component of thylacoidal membranes and especially, the polypeptide growth (6).

In the primary callus obtained in the MS(1962) without Spd may have seen a bordering of the total chlorophyll/carotenoid ratio (12.6) to the one obtained in the leaf (12.8).

The composition of the culture medium, light intensity (I = 3 000 lucs) as well as the daily light interval (16 hours light, 8 hours dark) have directly interfered with the calli influencing the cellular biosynthetic process. The plastids/cell number, their structural complexity correlates with the high chlorophyll and carotenoid pigments quantity.

The previous electron microscopically studies (4), on the leaf inocula from *Gardenia jasmoinoides* (Ellis) H. Br., cultivated on the MS(1962) culture medium added with spermidine in different concentrations (0.1 mM, 0.01 mM and 0.001 mM), have demonstrated that this species has reacted at the exogenous spermidine through the accumulation of large amilipherous inclusions, which have disturbed the structure and functions of chloroplasts. The massive accumulation of starch could by connected to the mesophyle resistance increase to CO_2 diffusion at the same time, to CO_2 assimilation rate reduction. This favorises the oxygenating activity in opposition to the carboxyl ting activity (7).

At the calli level, where the plastid system being weekly differentiated, we have observed the synthesis of a larger chlorophyll *b*- accessory pigment quantity, having the role of attracting the light energy, which, in its turn transmits it to the *a* chlorophyll.

The quantitative growth of the *b* chlorophyll and decreasing of the *a* chlorophyll, means that there would take place a plastid adaptation to the conditions imposed by the composition of the culture medium, as well as the weak light (3000 lucs and 16 hours/day light) with the purpose of attracting more light energy necessary to the fulfilling of photosynthesis.

The studies realized by Cheng (3), Dumitrescu (4,5) have evidentiated that different types of polyamines exogenously administrated have specific localization in the cell and influence in a different way the process of synthesis of assimilating pigments.

Spermidine is known to be an aliphatic polyamine, which interferes in the cellular metabolism regulation process (6).

Exogenously added to the culture medium in concentration of 0.1 mM, it caused some ultrastructural modifications, especially evident, on the plastids level of *Nicotiana tabacum* L. (cv. Xanthi), which are destroyed. The plastidial stroma occupied by a large number of amyliferous inclusions and the weak thylacoidal system has badly influenced the assimilating pigment synthesis process (5).

Gardenia jasminoides (Ellis) H. Br. reacted to the spermidine treatment through the accumulation at the plastids level of the large amilipherous inclusions and through the diminishing of assimilating pigments synthesis.

The total chlorophyll in the calli obtained on the MS(1962) culture medium added with spermidine, is halfway diminished (0.030 mg/1g s.p.), as compared to the total chlorophyll in the long-term calli (9) without spermidine (0.060 mg/1g s.p.). In the primary calli obtained on the standard culture medium MS(1962) without spermidine, the total chlorophyll has the value 0.38 mg/1g s.p. At the leaf level of the *Gardenia jasminoides* (Ellis) H. Br., the quantity of the total chlorophyll is superior to the long-term calli also to the primary calli obtained on the MS(1962) culture medium supplemented with spermidine (2.56 mg/1g s.p.). Next to Chaerle and Van der Straten (2000) also Maxwell and Johnson (2000)

quoted by Antofie (1), we consider that the expression pattern of a chlorophyll it can by utilized as marker of the detection of the different types of stress on the plants.

CONCLUSIONS

- *a* chlorophyll extracted and of the primary calli obtained on the MS(1962) culture medium, modified and added with Spd 0.1 mM is halfway diminished (0.011 mg/1g s.p.) as compared to the one extracted and of the long-term calli, obtained on the standard culture medium without spermidine (0.023 mg/1g s.p.) as to the one obtained and of the primary calli without spermidine(0.134 mg/1g s.p.).
- On all the three types of calli examined, the quantity of *a* chlorophyll is approximately six times lower as compared to the a chlorophyll extracted and of the leaf (1.51 mg/1g s.p.).
- *b* chlorophyll-accessory pigment exhibits values higher quantitative as compared to the *a* chlorophyll, on all examined type of calli.
- The highest values of the total chlorophyll have been registered at the level of primary calli obtained in the MS(1962) withouth Spd (0.38 mg/1g s.p.) also in the leaf (2.56 mg/1g s.p.).
- The lowest values of the total chlorophyll have been registered in the primary calli obtained on the culture medium (MS 1962) modified and added with Spd (0.030 mg/1g s.p.).
- The *a/b* chlorophyll ratio in all the types of calli studied is diminished in comparison to the same ratio, in the leaves.
- The calli obtained on the MS(1962) culture medium modified and added with Spd 0.1 mM contains the highest carotenoid quantities (0.006 mg/1g s.p.) compared to the other calli.
- The diminishing of the total chlorophyll/carotenoids ratio on the primary calli obtained on the MS(1962) culture medium modified and added with Spd (5), confirm that the apport of exogenous spermidine take quantitative diminishing of the assimilating pigments, as to the quantitative growth of the carotenoids, probably as a result of the modification of the components of the thylacoidal membranes.

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