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BRIEF COMMUNICATION

Chlorophylls and carotenoids in calli of sugar beet genotypes

B. KRSTIĆ*, S. MEZEI**, L. KOVAČEV** and S. PAJEVIĆ**

*Faculty of Sciences, Institute of Biology, 21000 Novi Sad, Yugoslavia**
*Institute of Field and Vegetable Crops, 21000 Novi Sad, Yugoslavia***

Abstract

Chlorophyll (Chl) and carotenoid (Car) contents in calli (hypocotyl, cotyledon, egg cell) showed statistically significant differences between sugar beet genotypes. Absorption spectra of extracts from green calli did not differ from those obtained from green leaves, while in "brown" calli the differences were caused by relatively high Car contents. Pigment contents in calli were significantly lower than in leaves.

Additional key words: absorption spectra, *Beta vulgaris*, dry matter.

In callus cells, synthesis of photosynthetic pigments depends on many factors: part of plant from which explants are obtained (Nakashima and Tsuda 1987), conditions of callus subculturing (Gaspar *et al.* 1988), mainly substrate characteristics (Nawa and Ohtani 1992, Crouch *et al.* 1993), and plant species (Krstić *et al.* 1992). The rate of pigment synthesis depends primarily on the rate of precursor synthesis (Nawa *et al.* 1993, Bisbis *et al.* 1994) and of chloroplast forming (Crevecoeur *et al.* 1992, Lavergne *et al.* 1992). The aim of our work was to determine the Chl and Car concentrations in calli of sugar beet genotypes.

Seeds were dissected out from pericarp and germinated on the Murashige and Skoog hormone-free media. Hypocotyl and cotyledon explants of sugar beet were inoculated on the Gamborg (B_5) media with 3 % saccharose. The medium was supplemented with 0.1 mg dm^{-3} gibberellic acid (GA_3), 1 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 mg dm^{-3} kinetin. The calli were subcultured three times at 30 d intervals.

The sample of fresh callus (0.5 g) was smashed in a mortar and extracted in 80 % (v/v) acetone containing bicarbonate (for pH stability) and quartz sand. The resultant suspension was filtered through a *G-4* funnel and made up to 25 cm^3 with acetone. Chl *a*, Chl *b* and Car contents were spectrophotometrically determined using the

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equations of Wettstein (1957). Absorption spectra of the acetone extracts were registered using the spectrophotometer *Beckman DU 60*. Three replicates were made and the results were statistically analyzed by the variance analysis, and the differences were tested at the significance level $P = 0.01$ (Steel and Torrie 1960).

Table 1. Chlorophyll and carotenoid contents [mg kg^{-1}(d.m.)] and dry matter [g kg^{-1}(f.m.)] in calli of sugar beet genotypes. Means with the same letter in each column did not differ significantly at $P < 0.01$.

Genotype	Explants	Chl *a*	*b*	*a+b*	*a/b*	Car	Dry matter
4nMM 93/S-29	hypocotyl	249^{a}	89^{bc}	338^{a}	2.80^{a}	165^{b}	85.8^{bc}
2nmm 93/96S	hypocotyl	92^{cd}	93^{bc}	185^{c}	0.98^{cd}	52^{e}	74.0^{d}
2nmm 93/S-77	cotyledon	58^{d}	110^{ab}	168^{c}	0.53^{d}	190^{a}	92.4^{bc}
Dana	cotyledon	68^{d}	34^{d}	102^{d}	2.01^{b}	52^{e}	92.0^{bc}
Dana	hypocotyl	121^{bc}	80^{bc}	201^{c}	1.51^{bc}	91^{d}	96.3^{b}
Hy-11	cotyledon	125^{bc}	69^{c}	195^{c}	1.86^{b}	115^{c}	114.4^{a}
Hy-11	hypocotyl	150^{b}	124^{a}	274^{b}	1.21^{bcd}	80^{d}	77.2^{bc}
2064 "n"	egg cell	237^{a}	78^{c}	315^{ab}	3.13^{a}	138^{c}	82.0^{bc}
LSD 1 %		42	20	53	0.68	23	14.6

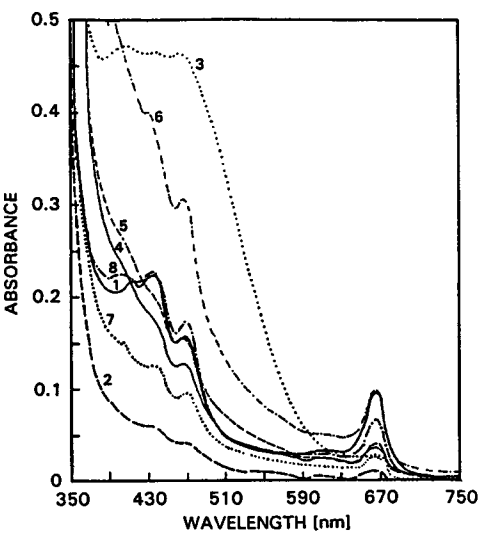

Fig. 1. Absorption spectra of photosynthetic pigments in calli of different sugar beet genotypes: (*1*) 4nMM 93/S29, hypocotyl; (*2*) 2nmm 93/96S, hypocotyl; (*3*) 2nmm 93/S-77, cotyledon; (*4*) Dana, cotyledon; (*5*) Dana, hypocotyl; (*6*) Hy-11, cotyledon; (*7*) Hy-11, hypocotyl; (*8*) 2064"n", egg cell.

After two weeks of culturing, the explants became highly swollen, and then nodular changes on the tissue surface were observed. Explants produced various amounts of calli, that differed in colour and texture. The time of callus appearance

differed between the eight tested explants. Multigerm line 4nMM 93/S-29 showed the earliest sign of primary green nodulous callus. Other tested genotypes expressed various colours from white, light yellow, brown to necrotic brown. The line 4nMM 93/S-29 had the greatest content of Chl, and the cv. Dana had the smallest one (Table 1). The quantities of pigments in tissue cultures were much smaller than in leaves of the same genotypes. Similarly, Lavergne *et al.* (1992) found in maize calli only 15-20 % of Chl content present in green leaves.

Habituated (hormone independent) calli may contain three times less Chl (*a+b*) than normal (auxin and cytokinin-requiring) calli (Gaspar *et al.* 1988).

Calli from the same line (Dana, Hy-11) contained more Chl (*a+b*) in hypocotyl explants than in cotyledon explants; differences of opposite character were found in Car contents.

Absorption spectra of green calli (Fig. 1) recalled the spectra of green leaves, while, in "brown" calli had relatively higher Car amounts. Also Nakashima and Tsuda (1987) found a similarity of Chl absorption spectra from sugar beet green calli and cotyledons.

High Chl *a/b* ratio may signalize high photosynthetic and yielding potentials of a given genotype (Watanabe *et al.* 1993). Low Chl quantity and low Chl *a/b* ratio may be due to low contents of tetrapyrroles (Hagege *et al.* 1992). Large variance in Chl *a/b* in our experiments (from 0.53 to 3.13 - see Table 1) could hardly be related to productivity.

References

Bisbis, B., Dujardin, E., Kevers, C., Hagege, D., Gaspar, T.: Chlorophylls and carotenoids in a fully habituated nonorganogenic callus of *Beta vulgaris*. - Biol. Plant. **36**: 443-449, 1994.

Crevecoeur, M., Hagege, D., Greppin, H., Gaspar, T.: Ultrastructural characteristics of cell from normal and habituated sugar beet calli. - Plant Physiol. Biochem. **30**: 87-95, 1992.

Crouch, N.R., Vanstaden, L.F., Vanstaden, J., Drewes, F.E., Drewes, S.E., Meyer, H.J.: Accumulation of cyanidin-3-glucoside in callus and cell-cultures of *Oxalis reclinata*. - J. Plant Physiol. **142**: 109-111, 1993.

Gaspar, T., Kevers, C., Penel, C., Crevecoeur, M., Greppin, H.: Biochemical characterization of normal and habituated sugarbeet calli. Relationship with anatomy, habituation and organogenesis. - Potsdamer Forsch. **57**: 20-30, 1988.

Hagege, D., Werckreichhart, D., Schmitt, P., Gaspar, T.: Deficiency in tetrapyrrole-containing compounds in a nonorganogenic habituated sugar beet cell-line. - Plant Physiol. Biochem. **30**: 649-654, 1992.

Krstić, B., Kovačev, L., Stanković, Ž.: Certain characteristics of leaf apparatus of different sugar beet line. - Contemp. Agr. **40**: 17-23, 1992.

Lavergne, D., Nato, A., Dupuis, J.M., Pean, M., Chagvardieff, P.: Evidence for the expresion of morphological and biochemical characteristics of C-3-photosynthesis in chlorophyllous callus-cultures of *Zea mays*. - Physiol. Plant. **84**: 292-300, 1992.

Nakashima, H., Tsuda, C.: Photosynthetic potential and growth of green cultured callus in sugar beet. - Mem. Fac. Agr. Hokkaido Univ. **15**: 169-172, 1987

Nawa, Y., Asano, S., Motoori, S., Ohtani, T.: Production of anthocyanins, carotenoids and proanthocyanidins by cultured-cells of rabbiteye blueberry (*Vaccinium ashei* Reade). - Biosci. Biotech. Biochem. **57**: 770-774, 1993.

Nawa, Y., Ohtani, T.: Induction of callus from flesh of *Gardenia jasminoides* Ellis fruit and formation of yellow pigment in the callus. - Biosci. Biotech. Biochem. **56**: 1732-1736, 1992.

Steel, R.G.G., Torrie, J.H.: Principles and Procedures of Statistics with Special Refference to the Biological Sciences. - McGraw-Hill Book Co., London 1960.

Watanabe, N., Ogawa, A., Furuta, Y.: Diversity of chlorophyll *a:b* ratio in durum wheat accessions and its association with photosynthetic rate. - J. Genet. Breed. **47**: 205-210, 1993.

Wettstein, D. von: Chlorophyll-letale und submikroskopische Formwechsel der Plastiden. - Exp. Cell Res. **12**: 427-433, 1957.