PHOTOSYNTHETIC ACTIVITY OF *in vitro* CULTURED *G. kurroo* Royle GERMLINGS MEDIATED BY SUCROSE CONCENTRATION

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Introduction

Somatic embryogenesis is the system of plant regeneration without any limitation in the production of germlings. Embryogenic cell suspension implantation on the agar medium, transfer to the regeneration medium and subculture of plantlets required a set of media supplemented with various combination and concentrations of PGR. Finally, the system produces plantlets which should be adapted to the *ex vitro* conditions. For tissue culture purposes the regenerants description usually concerns on type of growth, rooting, branching and size of leaf blade of already regenerated plantlets. These some extands depending on sucrose supplementing medium and photosyntetic apparatus activity. There are numerous parameters indicating how photosynthetic apparatus works: Fv/Fm (potential quantum yield of photochemistry), Y (yield - effective yield of photochemical energy conversion), qP (photochemical quenching), qN (non-chemical quenching), NPQ (implies part of non-photochemical quenching which is dissipated as heat), T (transpiration), Ph (photosynthesis), C (conductivity), CO_2 (CO_2 concentration) and RS (stomata resistance). The aim of this presentation is to show how sucrose concentrations in the medium affects function of photosystem of plantlets ready for *ex vitro* culture.

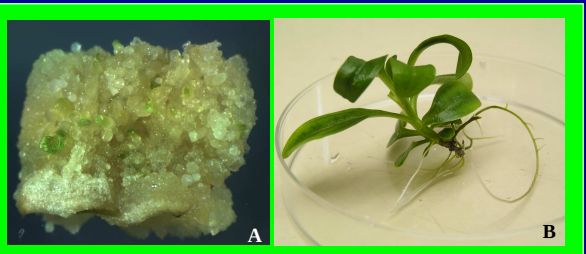


Fig. 1. Plant regeneration via somatic embryogenesis of *Gentiana kurroo* Royle initiated by the leaf blade culture
A. Somatic embryo induction on explant at the presense of MS medium suplementad with PGR
B. Germling used for studies on hormone free MS medium supplemented with sucrose

Tab. 1 Characteristics of 4-month-old analyzed germlings of *G. kurroo* originated from leaf – derived somatic embryos

No. combi-nation	Sucrose concen-tration (%)	Ratio of plant growth	Number of used leaves	Average of leaf mass (mg)		Average of leaf area (cm^2)
				fresh	dry	
1.	3.0*	-	4	41.1	5.4	1.7
2.	0.0	2.89	7	40.5	4.6	2.1
3.	0.1	3.42	6	55.5	4.9	2.6
4.	0.2	4.47	6	46.7	4.7	2.0
5.	0.3	3.11	7	27.6	3.4	1.2
6.	0.4	4.75	6	59.9	6.0	2.4
7.	0.5	3.35	6	40.3	4.7	1.7
8.	1.0	2.69	7	65.9	7.1	2.7

* control

Material and Methods

Experiments were carried out on the four – month - old plantlets of *Gentiana kurroo* Royle derived from somatic embryos regenerated from leaf blade explant cultures. Plantlets were cultured on MS medium supplemented with 0.0-10.0 g/dcm^3 and 30.0 g/dcm^3 sucrose (served as the control) (Tab. 1). For each sucrose concentration six to eight uniformed plantlets were studied. The analysis were done for four – seven leaves of one plantlets. The activity of photochemical process (light phase of photosynthesis) was determined by following parameters: Fv/Fm, Yield and quenching analysis using Photosynthesis Yield Analyzer MINI-PAM. Measurements were conducted on four leaves per plantlet on the central and apex area of leaf blade. Plant gas exchange (photosynthesis, transpiration, stomata resistance and CO_2 concentration) was measured also on four leaves usingwhole leaf blades by LICOR 6200. Data on fresh and dry matter, and leaf area were collected. Additionally, plant growth ration was counted (initial to final fresh metter) (Tab. 1).

Fig. 2. Potential (A) and yield photochemical effectivity (B)

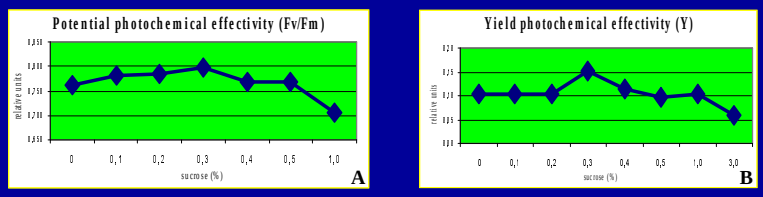
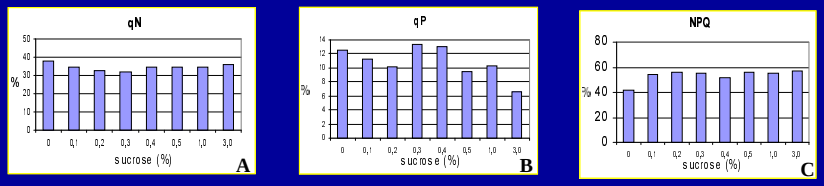


Fig 3. Participation of qN (A), qP (B) and NPQ (C) (%) calculated for the central part of leaf blade

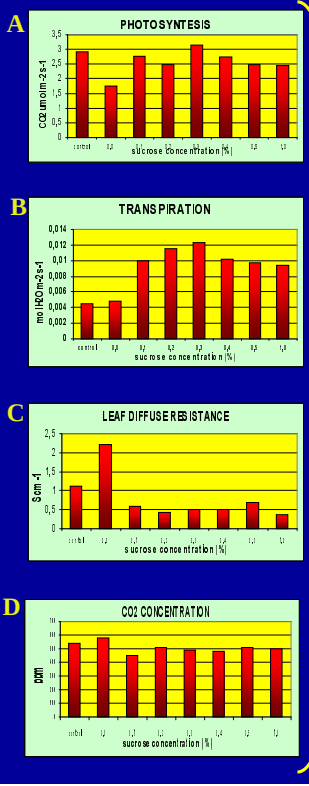


Results

The present work provides a multi-parameter approach to studies on gentiana plantlet photosynthesis. Vegetative propagation of gentiana required not only effective system for plantlet multiplication but system which should help in the transition from heterotrophy of somatic embryo to autotrophy of developed plantlet. For the description how the autotrophy of cultures is developed, several parameters of photosynthesis were selected for studies and described in the paper.

During four - months lasting culture, majority of studied plantlets reached multi-leaf stage of development (Fig. 1). The root system was not uniform and number of regenerated roots varied. The ratio of plant growth was different for each of studied sucrose concentration and varied from 2.69 to 4.75. (Tab. 1). The stage of plantlet development predisposed each of them for transferring to hardening culture and *ex vitro* condition.

The efficiency of the photosynthetic apparatus as measured by the ratio Fv/Fm, Yield and qP was highest when the medium was supplemented with 3.0 g/dcm^3 sucrose (Fig. 2 and Fig. 3). Potential of photochemical effectivity (Fv/Fm) reached level 0.800 of relative units what indicates that photosynthetic apparatus is developed (Fig. 2A). At this conditions effective yield of photochemical energy conversion was the highest (Fig. 2B). Chemical energy produced at the end of light phase and used for assimilation of CO_2 and Calvin cycle were the highest at 3.0 g/dcm^3 of sucrose in the medium (qP) (Fig. 3B). NPQ took the majority of light energy utilization in 3.0 g/dcm^3 sucrose cultures.



Among 7 studied concentrations 3.0 g/ dcm^3 of sucrose gave the highest photochemical efficiency which was almost two and half folds higher than control (Fig. 4A). At this concentration also the CO_2 absorption (dark phase) and transpiration rates were highest, and were associated with lower leaf diffusive resistance (Fig. 5). Although at highest concentration efficiency of the photosynthetic apparatus was lower, the CO_2 absorption was comparable to 3.0 g/dcm^3 sucrose. Also plantlets and their fresh weights were greater at higher concentrations but these were accompanied with higher diffuse leaf resistance and lower transpiration. Therefore, the better plantlets appearance and greater fresh weights might be due to higher water content.

Fig. 4. Forms of light energy utilization (A) and circular presentation only for 0.3% sucrose (B)

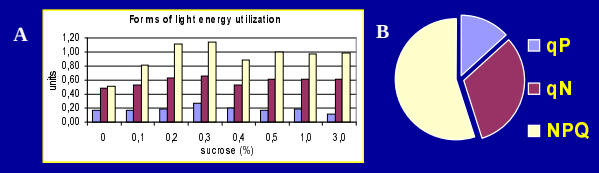


Fig. 5. Plantlet gas exchange (A) photosyntesis, (B) transpiration, (C) leaf diffuse resistance and (D) CO_2 concentration

We conclude that:

1) **3.0 g/dcm^3 of sucrose appeared to be the most suitable concentration for good development and functioning of photosynthetic apparatus of *Gentiana* plantlets and**
2) **there is no direct link between CO_2 absorption and sucrose concentration in the medium (in used in this study range)**

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