APPLICATION OF 2-D ELECTROPHORESIS FOR PROTEIN STUDIES OF PARTICULAR STAGES OF SOMATIC EMBRYO OF *GENTIANA KURROO*

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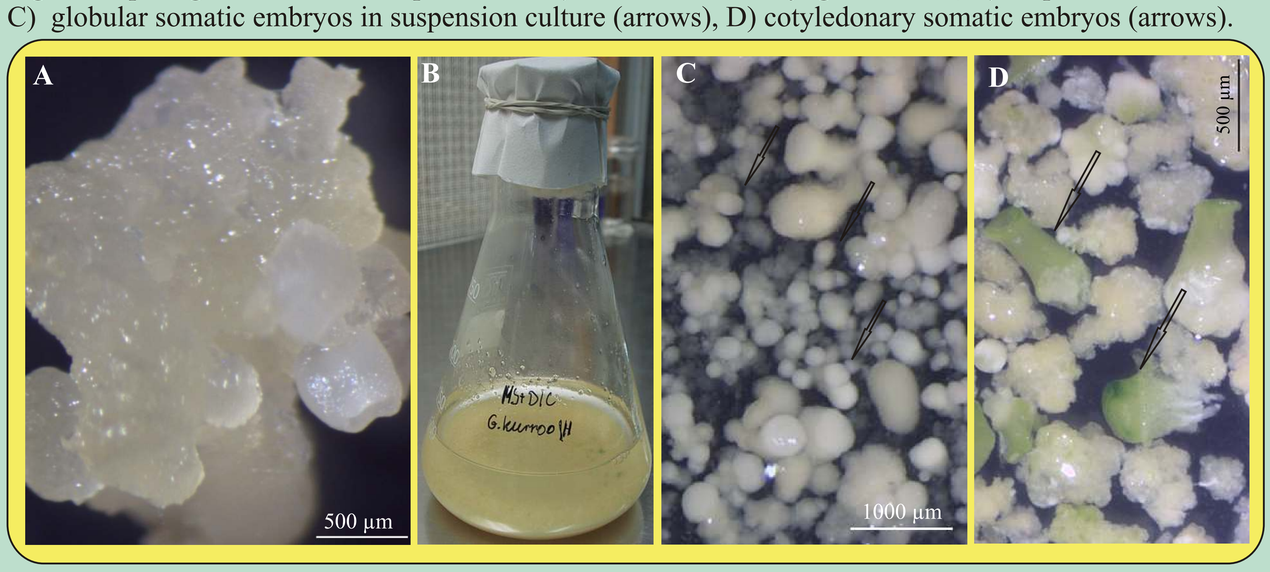
INTRODUCTION

Despite of nearly 50ty years which passed from discovery of somatic embryogenesis there is still not recognized until the end. So far, only few genes which regulate plants embryogenesis and only one isolated from somatic embryos - somatic embryogenesis receptor kinase (SERK), was menaged to describe (Fehér et al. 2003). The changes of genes expression which were detected with using of two - dimensional gel electrophoresis (2-DE) can constitute first step for gene identification which are directly connected with discussing processes. Very high embryogenic potential observed on primary explants and also suspension and protoplast cultures of *G. kurroo* (Fiuk et al.., 2003) cause, that this medicinaly important, protected by low species, present favorable object of experiments.

Fig.1. Preparing of material for experiments: A) induction of embryogenic callus, B) suspension culture, C) globular somatic embryos in suspension culture (arrows), D) cotyledonary somatic embryos (arrows).



AIM

The aim of this experiments was to show differences in the protein profiles of consecutive stages of somatic embryos isolated from cell suspension of *G. kurroo*. This analysis helps to investigate the changes which occurred in gene expression typical for the studied stages.

MATERIAL AND METHODS

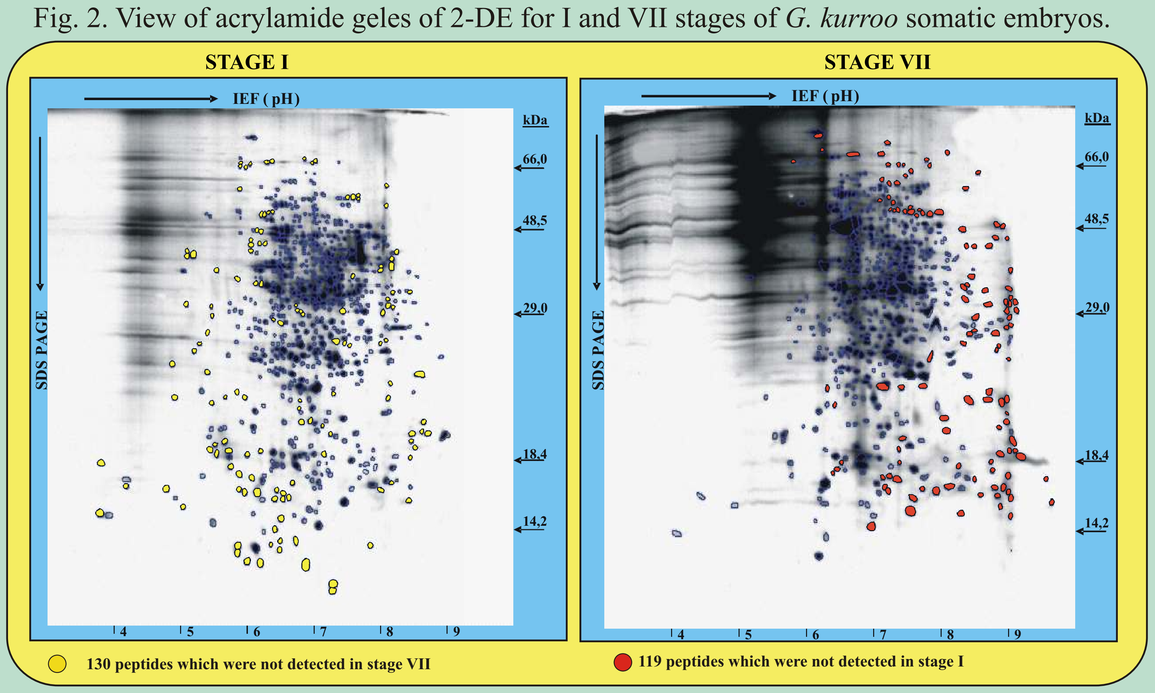
Seedling explants of *G. kurroo* were implanted on MS medium supplemented with 1.0 mg/l kinetin and 0.5 mg/l 2,4-D (Fig. 1A). Embryogenic callus which was appeared on them was used for suspension culture establishment (Fig. 1B). Somatic embryos in different stages of development (dependent of phase and size) were isolated from suspension and used for protein detection. Following stages were identyficated: I - globular embryos, II - late heart embryos with thick epidermis and differentiate rootlets, III - elongated embros with strongly designate rootlets, IV - cotyledonary embryos with closed cotyledones, V - cotyledonary embryos 2-3 mm in size, VI - cotyledonary embryos 3-4 mm in size, VII - cotyledonary embryos > 5 mm in size.

Protein extraction from 10 mg of tissue was performed according to the procedure proposed by Hurkman and Tanaka (1986), with application of extraction buffer (0.7 sucrose, 0.5 M Tris, 30 mM HCl, 50 mM EDTA, 10 mM KCl, 2 mM PMSF and 13 mM DTT) and resolving buffer (9 M urea, 4% Nonidet NP.- 40, 2%DTT, 2% Servalyte, pH 2-4). 2-DE was performed according to Hochstrasser et al. (1988). In the first dimension, isoelectrofocusing (IEF), a 1x150 mm capillary was used for polymerisation of a polyacrylamide column gel with Servalyte pH 3-10. IEF was conducted at room temperature for 18 h (50 - 1000 V). Proteins were separated in the second dimension (SDS-PAGE) in 13 % polyacrylamide slabs (1x150x150 mm). After electrophoresis, the peptides were stained with silver nitrate according to Heukeshoven and Dernick (1985). Protein spots on the gels were processed with Image Master 2-D Elite LKB software.

RESULTS

2-DE was applied for gentians for the first time and our experiments had also optimise meaning for this genus. However, the method let us to achive intended aim. On the base of acrylamide gels it was possible to assess molecular weight of detected proteins and differences between their expresion in particular stages. The molecular weight was varied from 12 to 70 kDa at presence of pH 4.0 to 10.0. We also observed the highest differences of protein dots between globular and cotyledonary stages. 2-DE show that 130 peptides which were characterystic for praembryos do not appeare in cotyledonary stage, while 119 characteristic for stage VII were not detected in stage I (Fig. 2 and 3). Late heart embryos (stage II) were represented by 96 proteins specyfic only for them (Fig. 3). Similarly for remaining stages: 50, 8, 66 and 23 specyfic protein were detected, respectively (Fig. 3). Cotyledonary embryos which were differed in lenght, also showed numerous changes. Lengthen of cotyledones (stage V, VI and VII) triggered a new genes expression, which were not active until now or blocked expresion of another genes. For cotyledonary stages from 8 to 64 dots did not appeare in next or previous stage.

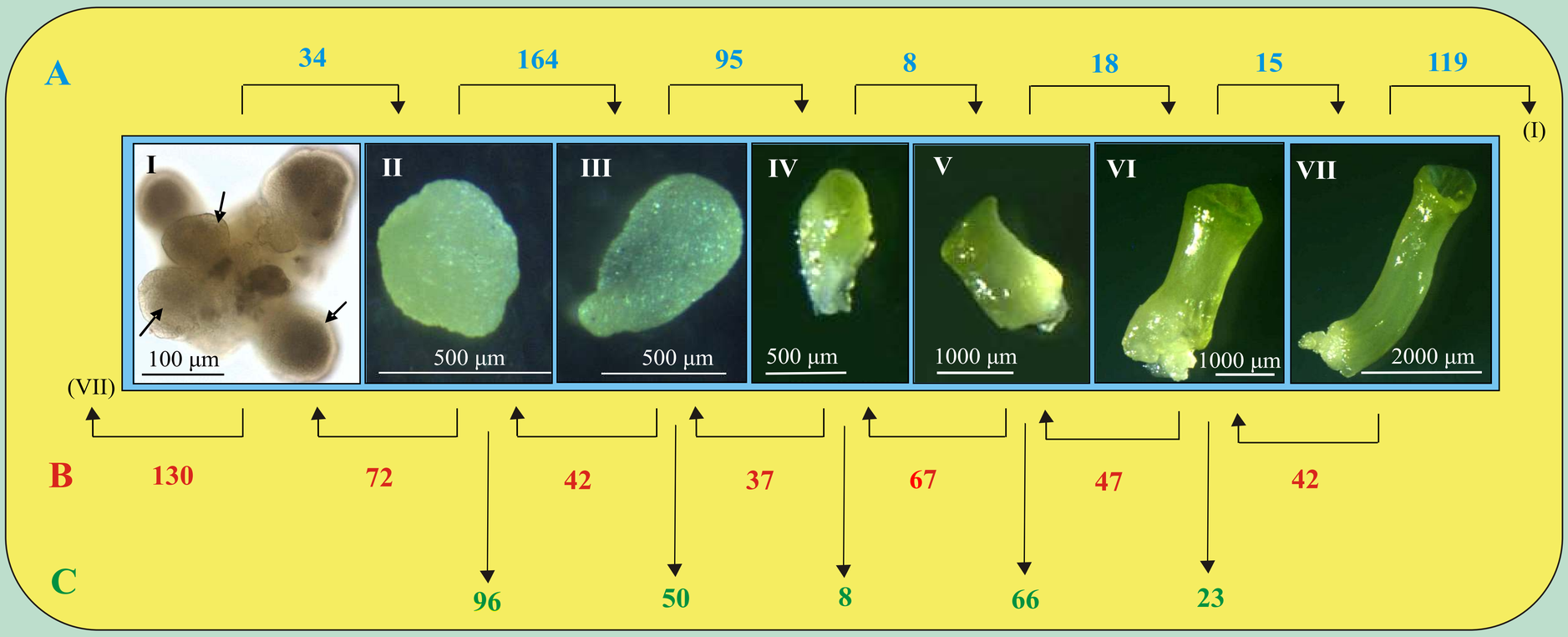
Fig. 2. View of acrylamide geles of 2-DE for I and VII stages of *G. kurroo* somatic embryos.



CONCLUSIONS

Two dimensional - electrophoresis in proposed procedure can be successfully use to demonstrate differences between particular stages of *G. kurroo* somatic embryo development.

Fig. 3. Number of protein spots for each investigated stage of development of *G. kurroo* somatic embryos: **A)** number of protein spots which were not observed with **next stage**, **B)** number of protein spots which were not observed with **precede stage**, **C)** number of protein spots characteristic for **current stage**.



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