TC-12 PLANT REGENERATION SYSTEM IN PROTOPLAST CULTURE OF *GENTIANA* GENUS

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Introduction: Long term studies on morphogenetical potential of various species of *Gentiana* genus revealed high morphogenic potential of their different tissues and organs. The obtained long-term cell suspensions characterised by embryogenic capability appeared to be basis for changing the level of experiments: from tissue to cell. Protoplast -plant cell without cell wall - in the sense of structure, organisation and function is the final subject of experiments concerning plant regeneration from single cell. The aim of the presentation was to work out a plant regeneration system initiated from cultured protoplast and completed by the culture of plantlet regeneration of *Gentiana kurroo* Royle.

Material and Methods: Protoplasts of fractioned suspensions were enzymatically isolated. Suspension originated from cotyledons and hypocotyl embryogenic callus cultured on MS medium supplemented with various PGR combinations. Three types of protoplast culture were employed: thin agarose layer, bead culture and liquid medium. The following media were used: MS, MS without NH_4NO_3 but supplemented with 3.0g/l glutamine and KM medium. All media were added with two sets of PGR typical for cell suspension culture and 9% of mannitol. Protoplasts were implanted with density $2x10^5$/ml. After 10 days of culture plating efficiency was scored. The osmotic preasure of the medium was decreased by medium changes once per week.

Results and Conclusions: The yield of protoplast isolation was dependent on the physiological condition of cell suspension and was scored 3.6-8.22 $x10^6$/ml for the studied cell suspensions. After 24hrs of culture the cell wall was formed. After 48 hrs the initial and sporadic division of protoplasts in agarose medium occurred. The highest plating efficiency was obtained when protoplast were cultured in the KM medium and MS media + glutamine. There were no significant differences between two studied PGR combinations and fractions of cell suspension. Plating efficiency was the highest for cultures maintained in a drop of agarose for cotyledon suspension protoplasts. After six weeks of culture protocallusses and embyoids in agarose media were observed. Three weeks later protocalluses and somatic embryos were subcultured on regeneration MS medium. Somatic embryos were only regenerated from cultures which originated from KM and MS without NH_4NO_3 and developed into plantlets within the next two weeks. Obtained plantlets were maintained on the 0.5 MS basal medium.