Conclusions

 In suspension cultures of tobacco BY-2 cell line derived from *calli* after transformation about 90 % of lines contained cells with various GFP fluorescence level after transformation.

 Newly introduced cloning method allowed obtaining nearly 50 % of clones with homogeneous *GFP* expression from primarily heterogeneous BY-2 lines.

 Heterogeneity of GFP expression in transgenic BY-2 lines had two causes - genetic (primary lines contained cells with different T-DNA insertions) and epigenetic one.

 Epigenetic heterogeneity of BY-2 lines was connected with transgene silencing, formation of stable epigenetic states early after transformation, and "permanent heterogeneity" with fluctuating levels in *GFP* expression.

 Reduction or silencing of transgene expression in potato was predominantly observed in lines with higher number of T-DNA insertions and with higher initial *GFP* expression.

 Silencing in potato always gradually affected both introduced genes. Silencing of *GFP* expression preceded (in months) loss of resistance to kanamycin (silencing of NPTII gene) in all monitored cases that indicates interconnections between silencing of both transgenes.

 The same sequence of silencing of both transgenes in potato was also observed in silenced lines after reactivation of transgene expression by 5-azacytidine, which induce DNA demethylation.

 Hypothetical four-steps mechanism for the gradual silencing of transgenes on the whole plant level was suggested: 1) posttranscriptional gene silencing (PTGS) of the *GFP* gene probably occurring accidentally in one or more cells of the plant; 2) spreading throughout the plant through siRNAs; 3) switch from PTGS to transcriptional silencing (TGS) through promoter methylation; 4) spreading of methylation into neighboring sequence *NPTII*.

 Analysis of 5-azacytidine inhibition effects on apical cuttings of potato plants allowed estimating 5-azacytidine half-life in cultivation medium to approximately two days.

 In both plant materials there has been an effective reactivation of transgene expression only after application of 5-azacytidine to intensively dividing cells.

 Combination of transient treatment of potato leave segments with 10 M 5-azacytidine and *de novo* regeneration on selection media with kanamycin was optimized to