Expression of the GFP gene in potato

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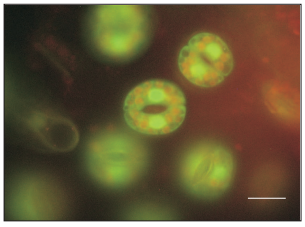
Introduction:

Green fluorescent protein (GFP) from *Aequorea victoria* has become a standard *in vivo* reporter in many biological systems. GFP can be also used as a transformation marker. Transformed tissue can be identified rapidly by UV or blue light illumination.

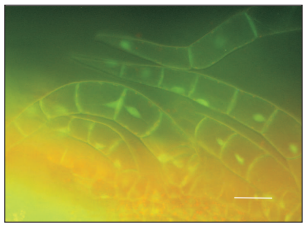
The original sequence of the GFP gene had to be modified for use in higher plants because of its aberrant splicing (Haseloff and Amos 1995) and protein insolubility (Davis and Vierstra 1998). Mutations in GFP fluorophore resulted in many different spectral forms (Tsien 1998).

Expression of the GFP gene like expression of other transgenes is affected by many factors. Differences in transcript level can be caused by transgene copy number, methylation state of genomic region into which the transgene has integrated as well as other environmental and endogenous factors.

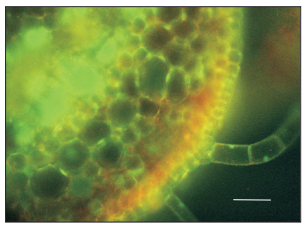
RS-GFP in potato



Stomatal cells (FITC filter cube)



trichomes (FITC filter cube)

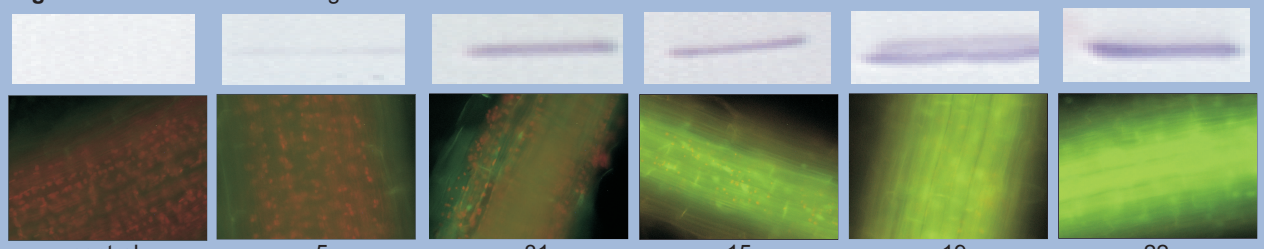


Stem cross section (FITC filter cube)

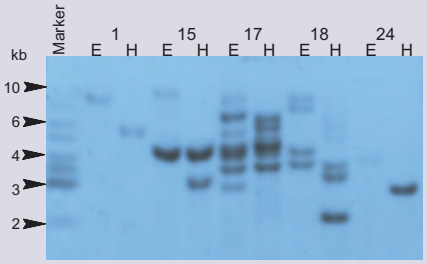
Both forms of fluorescent proteins used for transformation exhibited noticeable green fluorescence localised in cytoplasm and mainly in cell nucleus as introduced in Köhler (1998). Transformed plants expressing RS-GFP (pictures) showed 5-10 times higher intensity of green fluorescence than plants containing similar amount of common GFP (not shown). Thus only plants with RS-GFP were further studied.

Variability of the RS-GFP gene expression in transgenic potato lines

Fluorescence intensity of the RS-GFP in roots and **immunostaining on Western blot** were in a good correlation.



Southern blot analysis and hybridisation of genomic DNA with DIG-labeled *GFP* probe

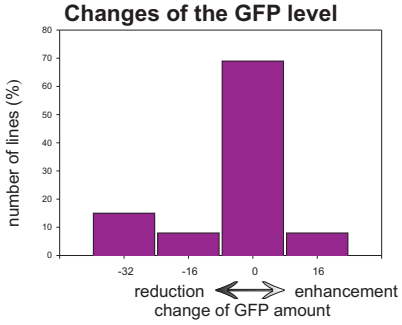


Genomic DNAs were digested by Eco RI (E) and Hind III (H) restriction enzymes . Southern hybridisation showed different numbers of transgene copies (from 1 to 7) in individual lines.

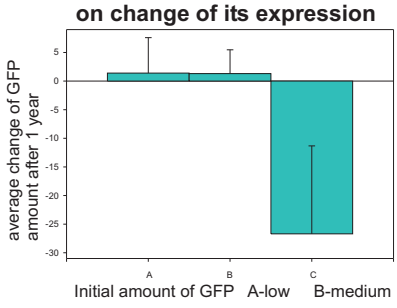
Stability of the GFP expression

Expression of the GFP was determined once again a year later.

Changes of the GFP level



Effect of initial amount of GFP on change of its expression



Expression of GFP stayed on the same level or decreased in the majority of lines. Initial higher levels of GFP often resulted in later reduction or complete loss of expression. GFP expression was not recovered during the process of de novo regeneration, which is conditioned by active expression of neighboring resistance gene. But in some clones, level of GFP was improved by 10 days-long treatment with 5-azacytidine (2mg/l).

Material and methods:

In vitro grown plants of potato (*Solanum tuberosum L.* cv. Désirée) were transformed by *Agrobacterium tumefaciens* according to Dietze *et al.* (1995) containing binary vectors with smGFP and smRS-GFP (Davis and Vierstra 1998). RS-GFP (red-shifted GFP) is a spectral variant of GFP characterised by 100 nm shift of excitation maximum into blue area of spectrum (Delagrave *et al.* 1995).

Microscopic examination of plants was done with an Olympus AX70 microscope fitted with standard FITC filter cube and special cube for detection of RS-GFP (U-MF2 Olympus).

Relative quantity of GFP in transgene plants was verified by Western blotting and immunodetection (Sambrook *et al.* 1989).

Transgene copy number in plants was determined by Southern blotting and hybridisation of genomic DNA with DIG-labeled *GFP* probe according to instructions of manufacturer - Boehringer Mannheim.

Conclusions:

- Due to high fluorescence intensity of RS-GFP, it can be used as a reporter gene in potato transformation.
- It must be taken into account that changes (mainly reduction) of transgene expression can occur in plants cultivated for longer period.
- There was no correlation found between the GFP gene copy number and level of its expression.
- Stability of transgene expression is partially influenced by the initial intensity of transcription.

References:

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