

TRANSIENT GENE EXPRESSION IN *OCIMUM BASILICUM* HAIRY ROOTS

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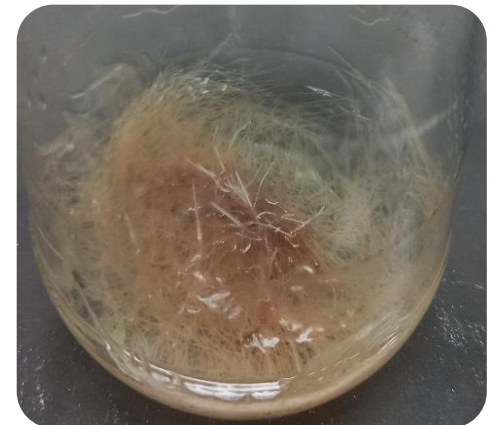
Transient expression in plants is a method to achieve target gene overexpression in a specific plant in a short time period (Nosaki S., Miura K. 2021) .

Hairy root (HR) culture, induced by plant infection with the bacteria *Agrobacterium rhizogenes* (now renamed *Rhizobium rhizogenes*), can be obtained from a wide range of plants and is a good alternative for plants under aseptic *in vitro* conditions.

Here we proposed a method for initiating the expression of foreign proteins using transient expression technology in HR culture obtained from basil (*Ocimum basilicum* L) leaf explants.

The aim of this study was to develop a transient expression technique in *O.basilicum* HR culture to produce green fluorescent protein (GFP) grown under *in vitro* conditions.

A stably growing HR basil culture was obtained on solid and liquid B5 medium.



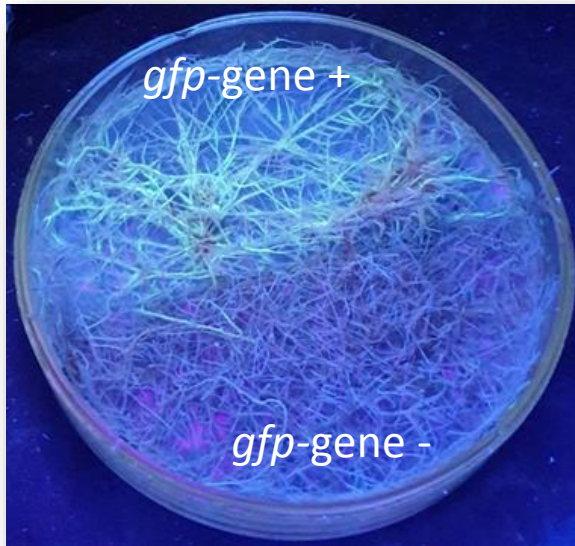


Fig 1. Green fluorescence indicating GFP accumulation 1 day after root infection

The HR culture was infected with agrobacterium suspension containing a plasmid vector with the *gfp-gene*. Roots were agroinfiltrated using a bacterial suspension applied on a filter. Negative control did not contain the *gfp-gene*. There was a 24-hour exposition with a bacterial suspension. Green fluorescence indicating on GFP accumulation appeared in infiltrated areas 1 day after root infection and was decreased for 5 days. Fluorescence under UV light was observed only on roots cultivated on solid agar medium. (Fig,2)

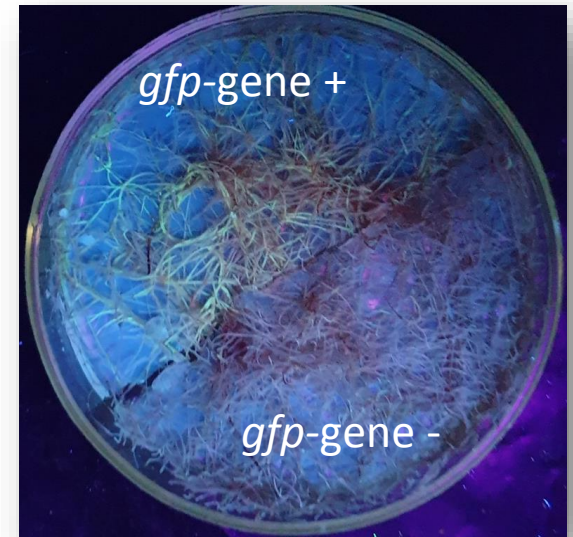


Fig 2. Reduced glow after 5 days

Liquid culture in a bacterial suspension showed the presence of bacterial contamination and the absence of a characteristic glow. Darkened roots do not express GFP (Fig.3)

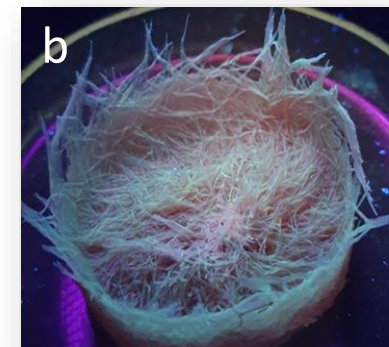
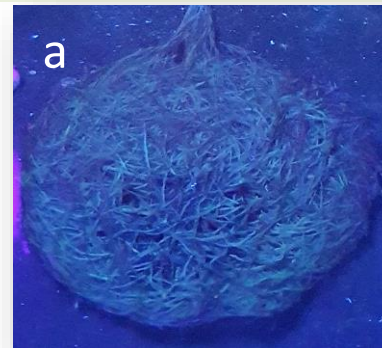


Fig 3. Hairy roots in liquid medium: a - *gfp-gene+*; b - *gfp-gene -* (control)

Conclusion: we demonstrated the ability of basil hairy root culture to express green fluorescent protein on agar medium. This study may provide an alternative method to potentially generate functional proteins in *in vitro* cell culture and will also promote further exploration of HR culture as a promising host for the production of recombinant proteins through transient gene expression.