Enabling tissue culture and gene transfer techniques for precise breeding in the local temperate rice Onix-INIA

***Marion Barrera*[1],** Blanca Olmedo[1], Carolina Zuñiga[1], Ricardo Vergara[1], Karla Cordero[2] and Humberto Prieto[1*]

[1]Biotechnology Laboratory, La Platina Research Station, INIA- Chile.
[2]Rice Breeding Program, Quilamapu Research Station, INIA-Chile.

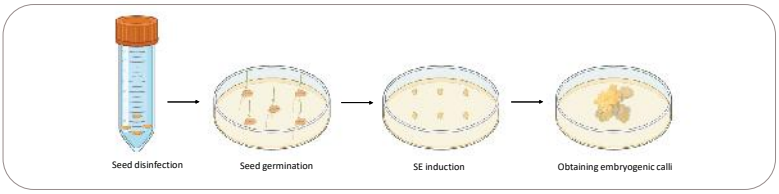
[*]Corresponding author: hprieto@inia.cl

ABSTRACT

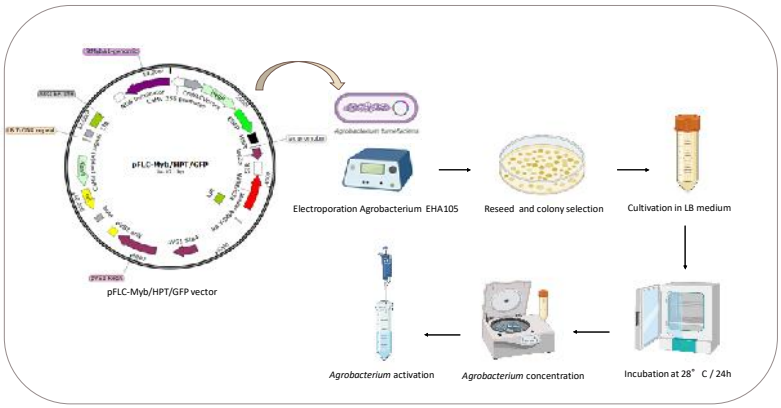
Rice (*Oryza sativa* L.) is one of the world's most important food crops and is a staple for more than half of the world's population. Generated by the local Rice Breeding Program and considered a new elite cultivar, "Onix-INIA" is the first black rice variety, adapted to temperate climate. Rice productivity is, however, conditioned by the effects of global climate change, leading to continuous limitations in yield potential and a decrease in arable lands that will likely impact production. The use of new breeding techniques supporting the genetic improvement of this species would allow for a more accelerated development of new varieties. Somatic embryogenesis (SE) is one base methodology for new plant breeding techniques, and particularly, in gene editing mediated by CRISPR-Cas technology. To enable GE in Chilean rice varieties, this research aimed the development of SE systems that allow for the transfer of CRISPR/Cas9 components in these genotypes. We assessed Agrobacterium-mediated gene transfer on somatic embryos of the elite variety Onix-INIA. Based on previous protocols, 2N6 medium was applied for seed germination and for embryogenic calli (EC) induction and propagation. Gene transfer on ECs was successfully achieved using the EHA105 bacterial strain harboring pFLC-Myb/HPT/GFP vector. The occurrence of the gene transfer process was monitored by visualization of the green fluorescent protein (GFP) expression through epifluorescence microscopy. The transformation events showed GFP-positive calli, as soon as the fourth day post infection. The regenerative capacity of hygromycin-resistant ECs was evaluated using MS regeneration medium (MSR). The resulting seedlings were subsequently transferred to a rooting medium (N6F). These results show that EC induction and propagation procedures in this study can be applied in genetic transformation and other biotechnology purposes in this variety.

Materials and methods

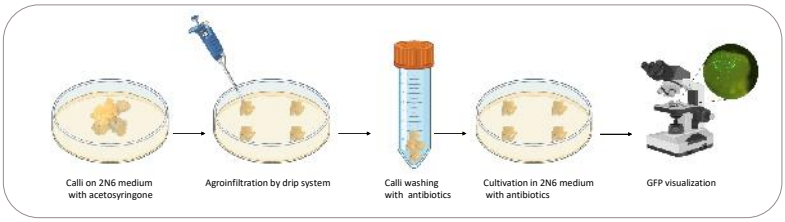
Somatic Embryogenesis



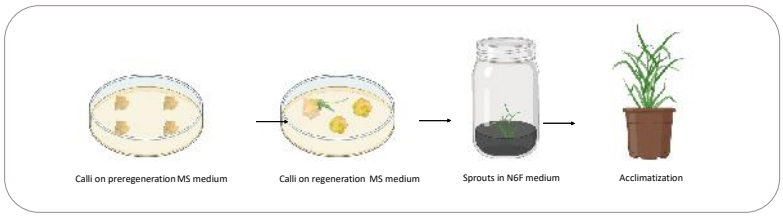
Preparation of *Agrobacterium*



Agrobacterium-mediated transformation of somatic embryos



Regeneration



Results

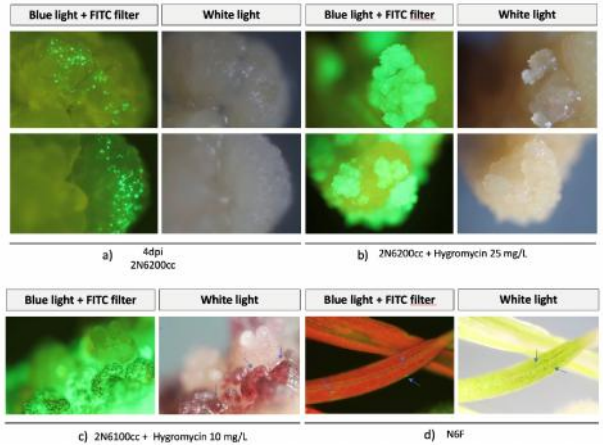


Figure 1. Evaluation of Agrobacterium-mediated gene transfer in Onix-INIA somatic embryos. a, Bacterial infections were performed at 15 d of primary calli and embryo masses cultured in 2N6 medium. GFP-emitting spots were observed in the calli as soon as 4 d post-infection. **b,** Cell masses showed stable GFP expression and were kept in this medium supplemented with cefotaxime and carbenicillin. **c,** Cell masses showed an expression of the marker gene StMyb. **d,** The leaves of the obtained seedlings showed stable GFP and StMyb expressions.

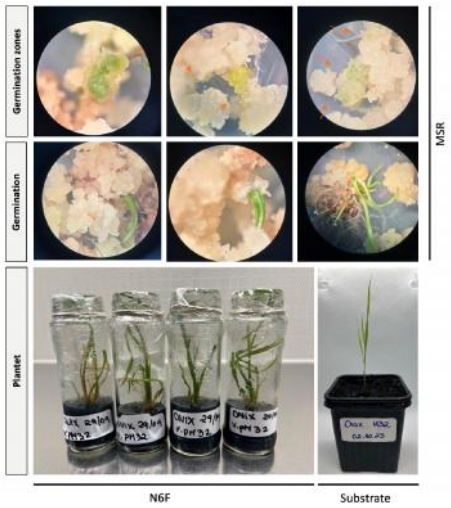


Figure 2. Regeneration of Onix-INIA GPF-positive calli. Callus germination was achieved by culturing embryogenic masses in MSR medium. Seedling development was achieved in N6F medium. The seedlings were placed in 3:1:1 peat, perlite and vermiculite for acclimatization.

Funded by grant Núcleo INIA 502955-70 (Humberto Prieto)