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Effect of the medium used in the growth of *Agrobacterium* strain on subsequent *gus* expression of the infected immature zygotic embryos of the tropical maize line

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The generation of stably transformed transgenic plants to assess gene function is a lengthy process. As an alternative, assessment of the transfer of transgenes into plant cells or tissues is often performed by use of transient gene expression assays. Transient *gus* expression systems are valuable tools for understanding the functions of genes in specific organs of plants.

Transient *gus* expression was carried out in the present study to test whether the constitution of the medium for growth of *Agrobacterium* had significant effect on the subsequent *gus* expression in the immature zygotic embryos of H627 maize line. Two different media were tested, namely, YEP (Yeast Peptone extract) and LB Luria-Bertani medium (LB) agar. EHA101(pTF102) *Agrobacterium* strain grown on these media was used to infect immature zygotic embryos of H627 maize line. The infection comprised of N6 salts, vitamins, 1.5 mg l^{-1} 2,4-D, 0.7 mg l^{-1} L-proline, 68.4 g l^{-1} sucrose, 36 g l^{-1} glucose and 200 µM Acetosyringone. The T-DNA region of the pTF102 vector contained *gus* reporter gene. The infected embryos were transferred onto the co-cultivation medium (N6 salt, vitamins, 1.5 mg l^{-1} 2,4-D, 0.7 mg l^{-1} L-proline, 30 g l^{-1} sucrose, 0.85 mg l^{-1} silver nitrate, 200 µM AS, 400 mg l^{-1} cystene and 3 g l^{-1} gerlite) and incubated in the dark at 20 ^{o}C for 3 days. Transient *Gus* activity studies were carried on immature embryos on the 3rd day of co-cultivation. Hundred embryos were stained for *gus* activity with 1 mM 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (XGluc) and 50 mM NaH_2PO_4 (pH 7.0) solution and incubated in the dark at 37 ^{o}C for 24 hours. The tissues were cleared using 70% (w/v) ethanol for 1-2 hours.

The gus expression was observed mostly at the edges of the infected immature zygotic embryos. The number and percentage area of the embryos which were stained blue due to transient *gus* expression was significantly ($p < 0.05$) higher when EHA101(pTF102) *Agrobacterium* strain was grown on YEP medium compared to LB agar prior to the infection of immature embryos of H627 (Fig. 1). This indicates that the type of the medium used for the growth of *Agrobacterium* strains prior to transformation influenced the transfer of the transgenes into the infected embryos.

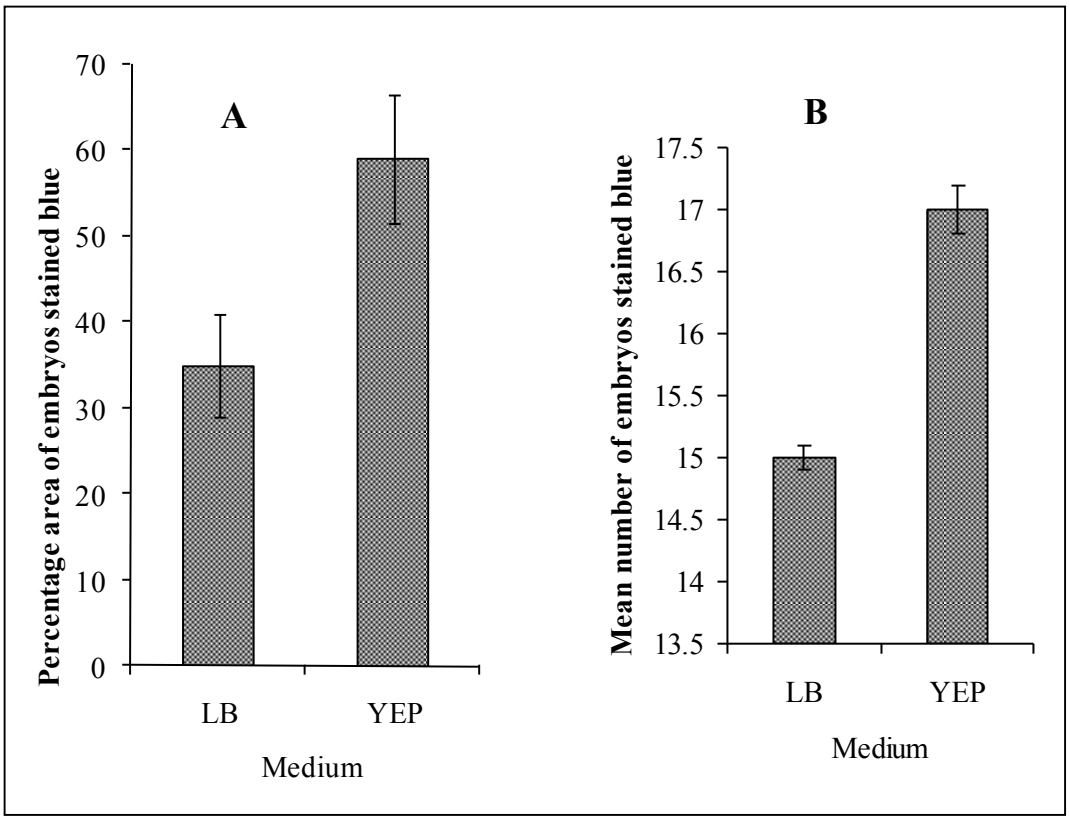

Fig 1. Immature embryos of H627 maize genotypes which showed *gus* activity when infected with EHA101(PTF102) pre-cultured in two different media before infection. A, Percentage areas of embryos stained blue; B, Mean number of embryos stained blue.