

**GENETIC TRANSFORMATION OF  
*LABISIA PUMILA* USING  
*AGROBACTERIUM TUMEFACIENS***

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## ABSTRACT

The present protocol was aimed to establish a routine transformation procedure via *Agrobacterium tumefaciens* for *Labisia pumila* var. *pumila*. The effect of different factors on T-DNA transfer by measuring transient expression levels of a disarmed strain LBA 4404 harbouring the binary vector pBI121 carrying chimeric glucuronidase (*GUS*) and neomycin phosphotransferase (*NPTII*) genes examined. Parameters optimized were light influence, types of wounding, types of explant, co-cultivation period, bacterial concentration, shaking influence, addition of glucose, pH, temperature, addition of acetosyringone and addition of sucrose. Improved transformation frequencies were obtained with an *A. tumefaciens* strain carrying kanamycin-type *vir* genes and when leaves were infected with *Agrobacterium* cells in the early log growth phase. Optimized co-cultivation was performed in the presence of 25µg/l of kanamycin. Methods used have expressed up to 77.62% positive transformants. This result was done using leaf explants co-cultivated for two days using a batch of *Agrobacterium* grown until giving the reading of 0.9 absorbance taken at 600nm wavelength. The leaves were poked using sterile needle and co-cultivated with *Agrobacterium* at room temperature with the addition of 0.02mg/l of acetosyringone, 5g/l of glucose and 15g/l of sucrose in the pH 7 medium. The mixture was left with 24h lighting and 100rpm agitation. Expression and presence of transgene was assayed by histochemical test and later confirmed with polymerase chain reaction. Seven percent transgenic plants were micropropagated and successfully acclimatized. The protocol is yet to be proven to enable it to be reproducible.