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Expression of Green Fluorescent Protein(GFP) Gene in *Nicotiana tabacum* cv. TI 516

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Objectives

In this study, we have conducted to establish an efficient regeneration system through leaf disc culture and identify the expression of GFP gene using *Agrobacterium*-mediated transformation method in *Nicotiana tabacum* cv. TI 516.

Materials and Methods

1. Establishment of efficient plant regeneration system in *Nicotiana tabacum* cv. TI 516
   a. Culture of leaf segments for 3 weeks on MS medium containing various concentration (0.1 to 2.0 mg/L) of PGRs (plant growth regulators : 2,4-D, Kinetin, NAA and BA)
   b. Examination of efficient PGRs on shoot regeneration from explants
2. Investigation of kanamycin concentration on MS basal medium with 0.1 mg/L NAA, 0.5 mg/L BA and kanamicin (0, 50, 100 or 200 mg/L)
3. Transformation of *Nicotiana tabacum* cv. TI 516 with A. *tumefaciens* strain LBA4404 containing a binary vector which carries the GFP gene
   a. Co-culture for 2 days on MS medium containing 0.1 mg/L NAA and 0.5 mg/L BA
   b. Transfer of explants on selection medium (MS medium with 0.1 mg/L NAA, 0.5 mg/L BA, 50 mg/L kanamycin and 300 mg/L cefotaxime)
   c. Selection of putative transgenic shoot on selection medium
   d. Plantlet formation on RT medium (MS medium with 50 mg/L kanamycin and 300 mg/L cefotaxime)
4. Genomic DNA isolation and PCR analysis with primer set (5'-GGG TCT AGA GGA GGG AAC CAT GAG TAA AGG AGA AGA AC-3'and 5'-AGC AAA GCA AAC ACC ATA TCC GAA AGT AGT G-3'), which are specific for GFP gene
5. Observation of the expression of GFP gene under fluorescence microscopy

Results and Discussion

1. The most efficient medium for plant regeneration was MS basal medium supplemented with 0.1mg/L NAA and 0.5 mg/L BA in *Nicotiana tabacum* cv. TI 516.
2. Kanamycin concentration for selection of transformant was 50 mg/L.
3. PCR analysis demonstrated that the GFP gene was stably integrated into the plant nuclear genome.
4. Expression of GFP gene in callus and plantlet have been identified by fluorescence microscopy.

Figure 1. Expression of GFP gene in callus(A), root(B) and apical meristem(C) of *Nicotiana tabacum* cv. TI 516.

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