Gene Insertion and Expression of a MybA1 Transcription Factor in *Nicotiana tabacum* (Tobacco)

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Nicotiana tabacum (tobacco)

- Tobacco is a model plant species in genetic transformation studies due to its ease in genetic manipulation.
- This characteristic makes it an ideal candidate for gene insertion and expression studies.
- Easily regenerated in tissue culture within 6-8 weeks

Agrobacterium–Mediated Transformation

Agrobacterium tumefaciens:

- It is pathogenic bacterium
- Genetically alters plant cells by transferring its DNA sequences (T- DNA), through wound sites, known as virulence genes that are present on the Ti plasmid.
- Transfer of T-DNA region is reached by activation of the virulence genes.
- At the site of infection will cause formation of crown galls.
- The T- DNA region responsible for gall growth is replaced by desired genes of study (mybA1).
- This T-DNA is part of the host genome and is expressed by the plant.

Objective

- In this study, a grape-derived MybA1 transcription factor that regulates anthocyanin pigment production was used as a reporter gene to study gene insertion and expression in tobacco cultures and plants.

Protocols

Preparation of Tobacco Explants

1. Around 100 seeds of cultivar ‘Samson’ were put in a 1.5 mL microcentrifuge tube and 1 mL of 2.5% sodium hypochlorite solution was added.
2. The surface of the seeds was disinfected by constant agitation on a shaker for 10 minutes.
3. The bleach solution was removed by washing 3 times, 10 minutes each with 1 mL of distilled water.
4. Seeds were transferred by evenly spreading them on petri dishes containing agar-solidified MS medium.
5. The petri dishes were sealed with parafilm and put in culture room at 25C, with a 18: 6 photoperiod.
6. Seeds germinated for 7-8 days,
7. Leaves from 3-4 week old seedlings were used as plants for transformation.

Protocols

Preparation of *Agrobacterium*

1. Agrobacterium containing the mybA1 gene under control of a constitutive promoter was cultured overnight
2. Antibiotics (carbenicillin, cefotaxime – 200 mg per l each and kanamycin – 100 mg per l) were dissolved in water and filter sterilized using .2 um nylon sterile filter, then used for culture growth
3. Culture was placed on a rotary shaker at 180 rpm and 28C.
4. After 24 hours the culture was centrifuged to collect bacterial pellet
5. Then the pellet was re-suspend in plant tissue culture medium (MS medium).
6. The culture was used for co-cultivation after 4 hours of shaking

Protocol

Agrobacterium-Mediated Transformation

1. Gathered 15 leaves from 2-3 week old tobacco seedlings
2. Wounded leaves using sterile tweezers
3. Transferred wounded leaves to a petri dish and added 5 mL of bacterial solution
4. Mixed thoroughly and let leaves suspend in the solution for 10 minutes
5. Removed leaves and blotted on a sterile filter paper
6. Then moved leaves upside down to petri dishes containing MST medium (MS medium containing BAP and NAA)
7. Left dishes in the dark for co-cultivation for 48-72 hours.
8. After co-cultivation blotted leaves on sterile filter paper and transferred 5 leaves upside down to 3 petri dishes containing MSTcck medium.
9. Grew for 3-5 weeks in light at 25C.

Protocols

- Shoot analysis:
  1. Individual shoots were detached from the calli and the total number of shoots was counted
  2. The total number of transgenic shoots in the Magenta boxes were identified on the basis of red color and counted
  3. This data was used to calculate success of transgenic shoot production

Results

Percent of transgenic shoot production from transformed leaf discs

$$\left(\frac{Number\ of\ transgenic\ shoots}{Total\ number\ of\ shoots}\right) \times 100 = \text{Percent transgenic shoot production}$$

Number of non transgenic shoots	Number of transgenic shoots	Total number of shoots	Percentage transgenic shoot production
62	21	83	25.3%

Results

Rooting

- The magenta boxes were collected after 2-3 weeks of growing and shoots that produced roots were counted

16/21 = Rooting

Number of Shoots	Number of none roots	Number of roots
21	5	16

Microscopy

- Scanning electron microscopy will be used to analyze differences in morphology and anatomy between transgenic and non-transformed plants.

- We plan on doing this in the future

Summary

- Genetic transformation is successful with MybA1 transcription factor, with Agrobacterium tumefaciens
- Can be quantified based on the appearance of anthocyanin pigment production

Thank You