Protocol to Examine the Effect of Antibiotics on Plant Morphogenesis

Method

1. Place transverse TCLs from stem internode tissue of in vitro 'Lineker' and 'Shuhou-no-chikara' chrysanthemum, as well as tobacco (light and dark) at 25 °C for a 16 hour photoperiod on an optimized shoot regeneration medium.

2. The shoot regeneration medium should contain 6 combinations of Cefotaxime [Cefotaxime Sodium Salt, GoldBio Catalog # C-104, (MW = 477.5 g/mol)], Carbenicillin [Carbenicillin Disodium, GoldBio Catalog # C-103, (MW = 422.36 g/mol)], and Vancomycin (Vancomycin Hydrochloride, GoldBio Catalog # V-200) at 50, 100, 200, or 400 µg/mL, as well as with or without antibiotic selection.

3. Make a new medium every two weeks. The shoots that can be derived from any medium should be harvested and placed onHyponex medium containing 20 g/L sucrose. The plantlets should be subcultured three times and maintained under 16 hour photoperiods at 25 °C.

4. The Chrysanthemum plantlets should be acclimatized and maintained in a greenhouse under LD conditions and placed in SD condition for flower induction.

References

Teixeira da Silva, J., & Fukai, S. (2001). The impact of carbenicillin, cefotaxime and vancomycin on chrysanthemum and tobacco TCL morphogenesis and Agrobacterium growth. *J. Appl. Hort* 3.1): 3-12.