A NOVEL PROTOCOL OF GENETIC TRANSFORMATION OF *Cuscuta campestris*

K.M.A.N. Alles[1], P.C.G. Bandaranayake[2] and T.D.C. Priyadarshani

[1]Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka.
[2]Agricultural Biotechnology Centre, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

Cuscuta spp. (Dodder) are agriculturally, biologically, and ecologically important holo-parasitic species worldwide. Nevertheless, molecular mechanisms of *Cuscuta* parasitism and their interactions with hosts are poorly understood. Though recent genomic and transcriptomic sequencing projects have identified candidate genes in *Cuscuta*, functional characterization of those genes has been hindered due to lack of stable genetic transformation system. This study developed a protocol of an *Agrobacterium rhizogenes* mediated transformation in *Cuscuta campestris* using pBIN-YFP vector with Yellow Florescence Protein (YFP) reporter gene. Three different culture media: Murashige and Skoog (MS) medium, MS medium containing 1 mgL^{-1} kinetin and 10% coconut water (K), MS medium containing, 1 mgL^{-1} benzyl adenine and 3 mgL^{-1} naphthalene acetic acid (MMS) were evaluated with three explant types and two co-cultivation periods at 15 °C in a three factor factorial study. Cut surfaces of apical and middle segments of three-day-old seedlings and wounded roots of one-day old seedlings were dipped in a 24h old bacterial lawn grown from MSU 440 strain harboring pBIN-YFP. Explants were co-cultivated on three media and maintained at 15 °C either for seven or fourteen days. Next the explants were transferred to the same media with 300 mgL^{-1} cefotaxime and maintained at 24 °C with 16 h light and 8 h dark intervals at 85 $\mu molm^{-2}s^{-1}$ intensity. The transformation efficiency was evaluated based on the percentage of explants with YFP expression. Of the factors considered only the media, explant and their interactions significantly contributed to the transformation efficiency. The apical explants cultured in MMS medium and maintained at 15 °C for seven days resulted 22% YFP expressing calli while middle explants cultured in the same medium maintained at 15 °C for fourteen days resulted 36% transient transformation. This protocol of transformation will set the foundation for gene functional characterization studies of *Cuscuta*.

Keywords: *Agrobacterium rhizogenes*, *Cuscuta*, Dodder, Genetic transformation, Parasitic plants, Yellow Florescence Protein (YFP)