DEVELOPMENT OF AN *Agrobacterium rhizogenes* MEDIATED TRANSFORMATION SYSTEM FOR *Lemna minor*

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ABSTRACT

Lemna minor is a model system for studying the genes responsible for plant growth under different water quality parameters and heavy metal absorption ability of roots. Though complete genome of *L. minor* is sequenced, further molecular works have been limited due to unavailability of efficient genetic transformation system. Therefore, the current research was focused on development of *Agrobacterium rhizogenes* transformation system for *L. minor*. Three different temperature ranges and light intensities were tested for growing of *L. minor* in 0.25 x Hoagland medium. Four different sterilization methods were tested, test 1; (0.5, 1, 2, 3, 5%) solution gradient from 14% v/v Clorox, submerged 8 min, test 2; 10% Clorox for 20 S and 70% ethanol for 20s, test 3; 70% ethanol for 20 S, followed by 1% Clorox 4 minutes and test 4; 70% ethanol and 4% of Clorox solution in 4-minute interval. Two different media (0.25x Hoagland and 1x MS), two explant types (sharply cut root surface and leaves) and three different infection methods (streak, liquid culture, centrifugation) were compared. *A. rhizogenes* strain MSU 440 harboring a vector with Yellow Florescence Protein (YFP) for selection of transgenic roots was used for the experiments. Transgenic roots were further confirmed by PCR with *A. rhizogenes* Ri plasmid specific rolB, rolC and VirD2 primers. According to the test results, 29-32°C temperature and 2466 and 4393 Lux light intensity are the most preferred. Out of the sterilization methods, most appropriate method is "test 2" which resulted 93%. In the transformation experiments, only the root-tip cut plants were survived and the transformation efficiency was higher in Hoagland medium than in MS medium. After eight weeks, around 17% of the plants infected with streak method, maintained in 0.25x Hoagland medium had at least one YFP expressing root. Both rolB and rolC genes were amplified from genomic DNA of YFP positive roots. These methods will be useful for future genetic studies of *L. minor*.