

Isolation of ACC synthase gene from *Striga gesnerioides*

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Abstract: *Striga* spp. are obligate parasites during early stages of growth and turn to hemiparasites on emergence. The parasite produces numerous minute long-lived seeds that don't germinate unless after-ripened, pretreated in warm, moist environment, and subsequently exposed to an exogenous germination stimulant that in nature is produced by roots of hosts as well as many non-host plants. Copious seed production and special germination requirements lead to build up of huge seed banks which constraint control measures and account for variability in their performance. Understanding the germination process in parasitic weeds may lead to development of novel control measures. The earliest detectable response to germination stimulants, in *Striga* species, is the production of ethylene. Germination stimulants elicit ethylene biosynthesis in *Striga* seeds and the ethylene produced induces germination. Ethylene is produced from methionine via S-adenosyl methionine, which is transformed into 1-aminocyclopropane-1-carboxylic acid (ACC). The enzymes involved in this pathway are ACC synthase and ACC oxidase. Germination stimulants break *Striga* seed dormancy by inducing ACC synthase and promoting ACC-oxidase activity. This study was conducted to isolate the gene that regulates induction of ACC synthase which in turn sets in motion the biological events involved in *Striga* germination. Conditioned *S. gesnerioides* seeds were induced to germinate by root exudates from hydroponic cowpea cultures. The resulting germlings were frozen in liquid nitrogen and total RNA was extracted. cDNA was synthesized, amplified and fragments encoding ACC synthase were obtained. The ACC gene was subcloned using T-Vector pMD20 (2,736 bp). Vector with plasmid inserts were collected. The vector cells, lysed, plasmid DNA was extracted and further amplified and checked by Agarose electrophoresis. Order of nucleotide bases in the inserted gene was determined by sequencing using two primers (primer M13RV and primer M13M4). Following a series of reactions, comprising of amplifications, separation and denaturation, the end product was analyzed using an automatic sequencer. The sequence obtained revealed that none of the clones had the target DNA fragment. The findings indicate failure to isolate the ACC synthase *Striga gesnerioides* gene. The failure to isolate the gene may be attributed to the non-specificity of the primers used. Very small fragment from *Striga gesnerioides* DNA has been characterized and that was used to design primers targeting the gene.

Keywords: *Striga*, ACC synthase, ethylene, germination, RNA, cDNA