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In-vitro uptake and localization of Cy3-labeled by *Setaria digitata*

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Setaria digitata resides in the abdominal cavity of ungulates and are generally nonpathogenic in their natural host, cattle. However, transmission of infective larvae into non-permissive hosts such as sheep, goats or horses can result in cerebrospinal nematodiasis (CNS). CNS is a neuropathological disorder that results in major dysfunctions of the central nervous system, producing symptoms of motor weakness, ataxia, lumbar paralysis and ultimate death of infected animals. Therefore, this disease poses a serious threat to livestock farming in tropical countries. Further, *Setaria digitata* can also infect humans and cause abscesses, allergic reactions, enlarged lymph nodes, eye lesions and lung inflammation showing gradual adaptation to humans. siRNA mediated RNA interference (RNAi) is considered to be a promising reverse-genetic tool to study gene functions. In this study, we used siRNA technique to specifically silence the expression of *S. digitata* novel parasitic nematode-specific gene (SDNG). SDNG has been proven to be specific for animal parasitic nematodes. It was found to be abundantly expressed in longitudinal muscles, reproductive systems and during embryogenesis, in all stages of the life cycle of *S. digitata*. siRNA was generated using the DNA fragments resulting from PCR amplification of plasmid vector containing *SDNG.* This was carried out using forward and reverse primer combinations (four primer pairs; SD1F/1R, SD2F/2R, SD3F/3R, SD4F/4R) containing T7 promoter sequences. dsRNA was synthesized using T7 RNA polymerase. They were cleaved to 21-23mer siRNA fragments using shortcut RNAse III, and labeled with Cy3 labeling reagents. siRNA so generated was used to treat adult worms (40µg/ml siRNA, 4hrs per day for 4 days) and microfilariae (10µg/ml siRNA, 3hrs per day for one day) in RPMI culture medium containing 10% FBS, 30 µg/ml Streptomycin, 2.5 µg/ml Amphotericin B without FCS in a CO_2 incubator in the presence of 5% CO_2 and at 37 ºC. The visualization of siRNA treated adult worms and microfilariae under the fluorescent microscope revealed uptake and localization of Cy3-labeled siRNA by *S. digitata* indicating that dsRNA is taken up by *S. digitata* which can be used for siRNA mediated RNA interference to study the functional role of *S. digitata* genes. This is the first demonstration of siRNA uptake by *S. digitata.*

Keywords: *Setaria digitata,* SDNG, siRNA

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