DEVELOPMENT OF RNAi BASED TECHNOLOGY FOR SWEETPOTATO VIRUS DISEASE RESISTANCE AND ITS CHARACTERIZATION IN *NICOTIANA BENTHAMIANA*

Tugume K. Joab (BSc. Agric) (MAK)

2010/HD02/670U

STUDENT NO: 203000824

A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF MAKERERE UNIVERSITY

DECEMBER 2012

ABSTRACT

Sweet potato virus disease causes significant losses of sweetpotato yields in many parts of the world and Uganda in particular. The disease is due to co-infection with *Sweet potato feathery mottle potyvirus* (SPFMV) and *Sweetpotato chlorotic stunt crinivirus* (SPCSV). While SPFMV causes characteristic mottling and ring-like symptoms, the most severe symptoms of the disease are caused by SPFMV in combination with SPCSV. It is difficult to conventionally breed for resistance to these viruses because of the complex nature of the sweetpotato genome. The main objective of this research was to develop transgenic technologies for enhancement of resistance to sweetpotato virus disease. The approach was based on the pathogen derived resistance, RNA interference (RNAi) in particular. RNAi based constructs were designed and characterized for biogenesis of small interfering RNA (siRNA) and control of SPFMV through a transient assay in *Nicotiana benthamiana.* Gene sequences targeting SPCSV and SPFMV were successfully fused and ligated into plasmid vectors to develop expression cassettes. Two fusion constructs were developed; one from regions of the viruses targeted most with siRNA and another one from regions of the viruses least targeted with siRNA. Transient expression studies in *Nicotiana benthamiana* and subsequent Northern blot analysis showed that the developed gene constructs can be transcribed and processed into siRNA. Constructs designed from regions of the virus most targeted with siRNA produced more siRNA compared to a construct designed from regions of the virus least targeted with siRNA for both viruses. A transient challenge and expression of resistance against SPFMV showed that the constructs could protect *Nicotiana benthamiana* plants against SPFMV. A construct designed from regions of the virus most targeted with siRNA (HT-127) produced better transient resistance against SPFMV than that from regions of the virus least targeted with siRNA (LT-227). From the above, it is likely that when the constructs are used for stable transformation of sweetpotato the plants will be protected from natural infection with SPFMV.