



**ROSETTE VIRUS DISEASE COMPLEXES ASSESSMENT AND DEVELOPMENT OF  
GROUNDNUT REGENERATION PROTOCOL IN UGANDA**

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## SUMMARY

Groundnut Rosette Disease (GRD) caused by a complex of GRAV (groundnut rosette assistor luteovirus), GRV (groundnut rosette umbravirus), and the allied satellite RNA (sat-RNA) is the leading production constraint causing total yield losses in unsprayed susceptible varieties. This study determined and geo-referenced the distribution of the GRD symptom types in Uganda, characterized viral complex components causing GRD in Uganda, and developed a regeneration protocol for Ugandan preferred cultivars.

A nationwide survey covering 23 districts was done in June and July of 2013 to ascertain and geo-reference the distribution of the GRD symptom types. For viral complex characterization, 22 geo-referenced groundnut samples were collected (from both GRD infected and healthy plants), RNA extraction, cDNA synthesis, PCR amplification, electrophoresis, staining and visualization were performed according to standard procedure. Regeneration protocol development involved use of embryo axes explants from freshly harvested mature seeds representing Spanish, Virginia and Valencia groundnuts botanicals initiated on Murashige and Skoog (MS) basal media with varying concentrations of the growth regulator 2,4-Dichlorophenoxy acetic acid (2,4-D); Chu N6 basal medium with vitamins (N6); and Callus Induction Medium (CIM). The shoot and root formation media contained supplemented MS basal medium.

Data were analysed using SPSS, chi-square test, and Arc-GIS for mapping GRD severity scores. Results showed that Uganda is a green rosette belt with two hotspots identified at Nakabango and Serere in Eastern Uganda. The GRD agents occurred in space and time attributed to aphids feeding behaviour and the optimised RT-PCR detected all the GRD complexes in both symptomatic and asymptomatic samples. The groundnut genotypes representing the three botanicals responded differently to media compositions for both callogenesis and regeneration. The use of readily available mature seeds embryo ex-plant gave rapid yields for both callus and plantlets. This study recommends that Nakabango and Serere be GRD primary selection and breeding sites. Use of multiplex PCR for simultaneous detection of all three viral pathogens in one PCR reaction mixture will provide a cheap, rapid assay thereby expanding the scope for GRD resistance screening. Genotype specific protocols are needed since genetic background and media composition had effects on both callogenesis and regeneration. The use of dry seed embryo axes explant gives high callus and plantlet yields in addition to assuring year-round explants availability for continuous research.