Abstract

In Uganda, groundnut (*Arachis hypogaea* L.) is the second most important staple legume after common beans, grown majorly by smallholder farmers for energy, protein, essential vitamins and oils, food, feed and income. Current production can fetch $344 million annually to the Ugandan economy. 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 constraints causing total yield losses in unsprayed susceptible varieties. Host plant resistance offer the best control mechanism in terms of costs for the farmer and environmental safety. The general study objective was to contribute towards increased groundnut production in Uganda by generating knowledge on the GRD and potential management options. Specifically, the 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 2012 and 2013to ascertain the predominant GRD symptom, GRD incidences and severity, farmers' knowledge and GRD managements, current seed systems and farming practices. Data were analysed using SPSS and chi-square tests. Mean GRD severity scores were geo-referenced and plotted on the Uganda map.

Twenty two groundnut samples were collected from both GRD infected and healthy plants and sites geo-referenced. RNA extraction, cDNA synthesis, PCR amplification, electrophoresis, staining and visualization were performed according to standard procedure.

Embryo explants from freshly harvested mature seeds representing three groundnuts botanicals (Spanish, Virginia and Valencia) were initiated on 3 media; 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 formation and elongation medium contained MS basal medium supplemented with indolebutyric acid (IBA) and 6-Benzylamminopurine (BAP) in isolation, and BAP in combination with a-naphthaleneacetic acid (NAA) and indoleacetic acid (IAA). Elongated shoots were transferred to MS medium supplemented with various NAA combinations with IBA, BAP and a combination

of IBA and Kinetin for root induction.Data were collected on the number andpercentage of callus formed, days to callus formation, number of ideal calli, shoot and root formation conditions. Dataset was subjected to analysis of variance using Genstat 14th Edition. The cultivars and concentration were ranked using Kruskal-Wallis one-way test.

The results from GRD distribution and characteristics study showed that current farmers' GRD knowledge did not have a significant effect on its management, seed sources and varieties grown. The green rosette type was the most predominant making Uganda a green rosette belt. Two hotspots for groundnut rosette virus (GRV) were identified in Eastern Ugandaat Nakabango and Serere for primary GRD screening and breeding.

Groundnut samples showed combinations of GRD agents, some in isolation, combination and presence of all three agents signifying that separation of the GRD agents occurs over time and space. Both green and chlorotic GRD symptoms were observed indicating presence of sat-RNA variants. The RT-PCR technique detected the GRD agents both symptomatic and asymptomatic samples showing robustness of this method over phenotypic screening.

This research successfully regenerated reproducible complete plantlets from embryo axes segment via embryogenesis providing the essential prerequisite for transgenic GRD research. Groundnut cultivars showed significant divergence for *in-vitro* response to ideal callus induction and the subsequent regeneration suggesting that genetic factors are primordial in the determination of *in vitro* tissue culture response level.

This study recommends that the current GRD extension system needs to deploy the knowledge about the disease into practical management options. Nakabango and Serere hotspot sites should be GRD primary selection and breeding sites. Necessity for routine GRD symptom types documentation in Uganda as symptom types shift have been reported. Phenotypic screening needs validation with molecular tools in efficient diagnosis of the multi-pathogenic GRD to guide breeding and pathology works.The use of dry seed embryo axes explant guarantee year-round explants availability for continuous research. Genotype specific protocols are needed since genetic background and media composition had effects on both callogenesis and regeneration.