Regeneration procedure for three *Arachis hypogaea* L. botanicals in Uganda through Embryogenesis

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Introduction: This study explored the use of mature embryo axes as explants for somatic embryogenesis and determined the factors that affect regeneration of three Ugandan groundnut cultivars Serenut 4T (Spanish), Serenut 1R (Virginia) and Acholi-white (Valencia).

Methodology: Freshly harvested mature seeds of three groundnut cultivars were collected and the embryo explants (**Fig.1**) were initiated on 3 media namely; 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). For root induction, elongated shoots were transferred to MS medium supplemented with various combinations of NAA with IBA, BAP and a combination of IBA and Kinetin.

Results: Different concentrations of 2,4-D elicited different callogenesis responses in the cultivars. Acholi white (Valencia botanical) and Serenut 4T (Spanish botanical) gave optimal response at 5mg/l whereas Serenut 1R (Virginia botanical) showed best response at a concentration of 30mg/l. N6 and CIM supported callogenesis in Acholi white (AW) and Serenut 4T only (**Fig. 2**). In all cultivars, maximum root production was gained when using MS medium supplemented with NAA-1mg/l and IBA -2.0 mg/l. On the other hand, for Serenut 1R and Serenut 4T, BAP 2.5mg/l; NAA 0.5mg/l combination yielded higher shoot regeneration percentage whereas for AW BAP 3mg/l; NAA 0.5mg/l supported maximum shoot production

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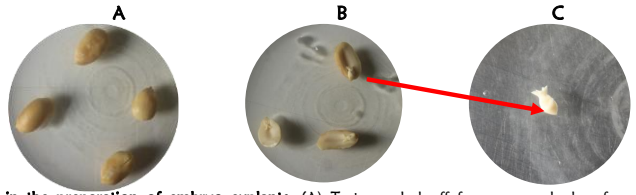

Fig. 1. Stages in the preparation of embryo explants; (A) Testa peeled off from pre-soaked surface-sterilized seed, (B) Embryonated cotyledon, and (C) Embryo axis aseptically excised by removing the cotyledon (arrow) and used as explant.

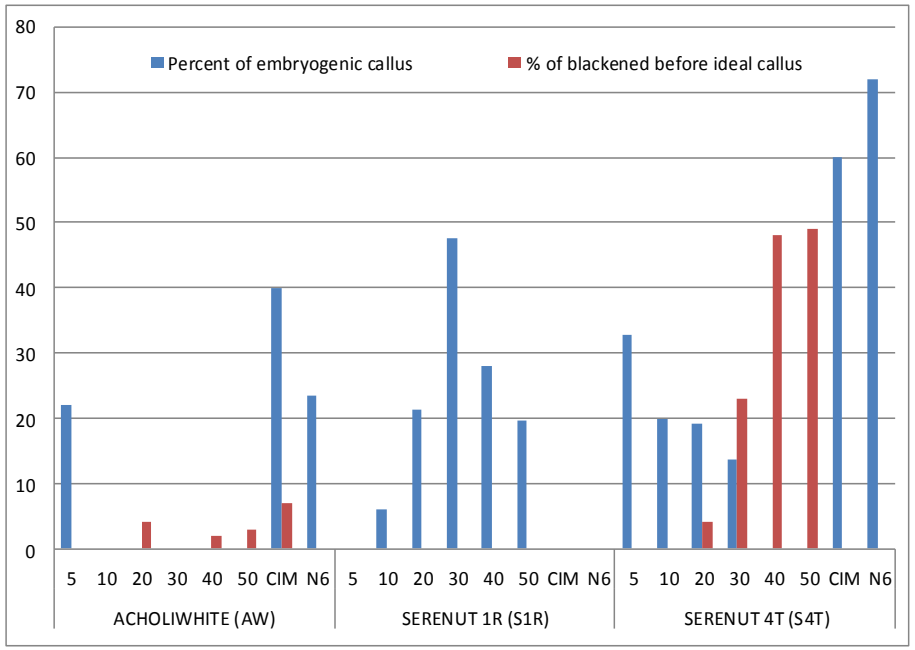

Fig. 2: Effect of groundnut cultivars used, media, and media concentrations on callus formation.

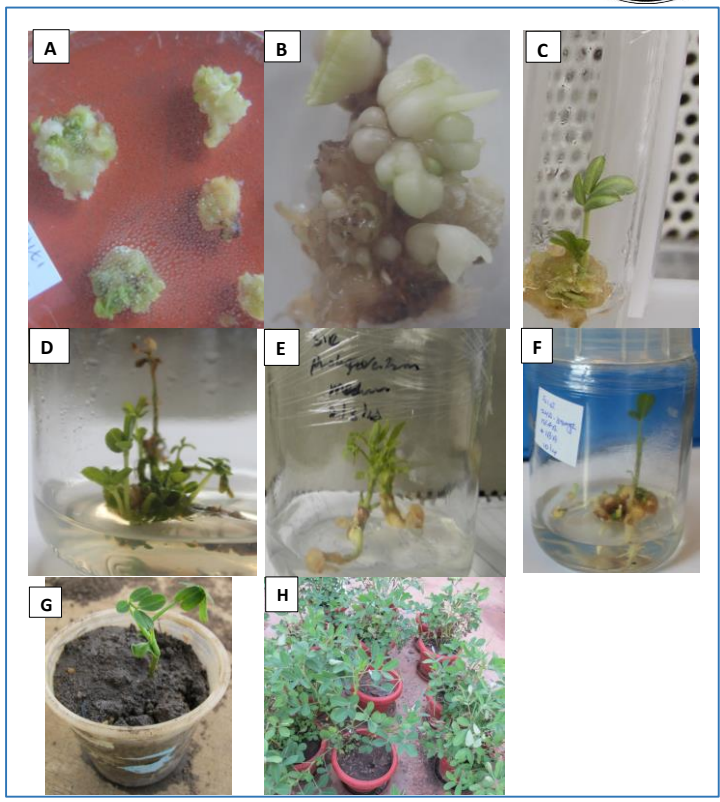

Fig 3. Multiple shoots and root developmental steps from embryo explants. (A) Embyogenic callus formed after incubating the embryo explant (B) Leaf shoot primordia growing from ideal callus (C) Fully formed leaflets growing on MS medium supplemented with BAP-3mg/l and NAA-0.5mg/l (D) Shoot cluster proliferated and maintained on MS medium supplemented with BAP-3mg/l and NAA-0.5mg/l. (E) Elongated shoot on rooted on MS medium supplemented with NAA- 1mg/l and IBA -0.5mg/l. (F) Rooted shoot on MS medium containing 4.95 mMNAA. (G) Tissue culture plant acclimatizing in a plastic pot. (H)Well adapted and matured plants in the glasshouse

Conclusions: This study optimized regeneration protocol for three groundnut cultivars in Uganda representing the Spanish, Virginia and Valencia botanicals via embryogenesis (**Fig.3**)

- Significant divergence for their *in vitro* response to ideal callus induction and the subsequent regeneration exhibited suggests that genetic factors are primordial in the determination of *in vitro* tissue culture response level
- When all conditions are optimal for the cultivars' regeneration, peak ideal callus formation takes 42 days from initiation in three media tested.
- The usages of mature dry seeds as explant source guarantee year-round availability of explants for continuous research.
- This protocol can still be improved further to increase the percentage of the regeneration with the potential of enhancing exogenous gene transfer through genetic engineering in the near future.