**Characterisation of Defensin-Like peptides (DEFL) family members involved in cowpea reproduction**

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Cowpea (*Vigna unguiculata [L.] Walp*.) is a valuable legume crop, but its yields are often suboptimal, particularly in regions like sub-Saharan Africa. Effective communication between male and female reproductive tissues is essential for successful plant reproduction and seed formation. Recent evidence from studies in *Brassicaceae sp.* and maize indicates that small, secreted Defensin-Like (DEFL) peptides act as signaling molecules in these critical interactions [1]. However, in cowpea and other species, gene annotation and functional understanding of these peptides remain limited [2]. Using bioinformatics, the aim is to identify the complete family of cowpea genes encoding DEFL peptides and pinpoint candidate genes acting in plant reproduction. Two highly and specifically expressed DEFL genes in the female embryo sac before and after fertilization have been identified. Validation of DEFL1 and DEFL2 gene expression was conducted by qPCR analyses of pre and post-fertilization whole gynoecia samples. Additionally, cowpea was transformed with a nominal 3Kb portion of DEFL1 and DEFL2 promoters fused to ZsGreen fluorescent protein. However, results suggest that the selected promoter regions lack critical elements. RNA in situ hybridization assays is currently being performed to examine DEFL1 and DEFL2 mRNA localization within the ovule. Finally, DEFL1 and DEFL2 knockout mutants were successfully developed through plant transformation using CRISPR-Cas9. T1 generated mutants are currently growing in our glasshouse facilities, awaiting flowering for phenotypic analysis. Through microscopy, we aim to determine whether these mutant plants can undergo normal fertilization and normal seed development.

***References:***

[1] Kim, M-J. et al. (2021). Peptide Signaling during Plant Reproduction. Trends Plant Sci. 26(8), 822-835.

[2] Feng, YZ., Zhu, QF., Xue, J. et al. (2023). Shining in the dark: the big world of small peptides in plants. aBIOTECH 4, 238–256.