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FUNCTIONAL ANALYSIS OF CYCLIN D2;1-TYPE GENES EXPRESSED IN TRANSGENIC BANANA PLANTS

By

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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Signed.....

PROF. KARL KUNERT

DEDICATION

To my wife Susan Talengera, daughter Norah Nampiima, and sons Andrew Talengera and Simon Mukoka.

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SUMMARY

Early maturity is one of the most important aerial growth traits next to bunch size, in determining banana productivity. However, low seed fertility in banana and the lack of breeding lines limit the application of conventional breeding for this trait. Genetic transformation with a *CyclinD2*-type gene, responsible for the CyclinD2 protein sub unit, which modulates the cell cycle progression at the G1/S phase, enhanced growth of tobacco plants. On this basis, investigations were carried out on the possibility of increasing the growth rate of banana plants through transformation with and expression of a *CyclinD2*-type coding sequence. *Arabidopsis thaliana;CyclinD2;1* (*Arath;CycD2;1*) gene and its banana ortholog were over-expressed in banana to test their growth enhancement potential. The banana *CyclinD2;1* (*Musac;CycD2;1*) was isolated from an East African highland cooking banana (AAA) cultivar 'Nakasabira' and a cDNA was created by PCR using degenerated primers which was followed by genome walking. Characterization of the banana cyclin protein revealed an IWKVHAHY motif that was found to be conserved across the Musaceae family. Phylogenic analysis revealed a higher protein sequence identity of this banana cyclin to *CyclinD2;1* of monocot plants than that of Arabidopsis. This cyclin was also found to be expressed highly in meristematic tissue which linked it to the cell cycle. The coding sequence was submitted to the GeneBank under accession number HQ839770. Arabidopsis and banana *CyclinD2;1* gene coding sequences under the control of a constitutive promoter were used to transform embryogenic cells of the banana cultivar 'Sukalindiizi' (AAB) using the Agrobacterium transformation system. A higher relative expression of *Arath;CyclinD2;1* was found in the shoot than in the root apices and expression reduced transcript amounts of the endogenous banana *CyclinD2;1*. Plants of transformed banana line D2-41 had the highest *Arath;CyclinD2;1* transcript amount and exhibited a significantly faster leaf elongation rate, better root growth, faster first leaf opening and a bigger lamina composed of bigger epidermal cells than non-transformed control plants. Banana plants transformed with *Musac;CyclinD2;1* had a higher transcript amount of the transgene in the root apices when compared to the shoot apices. The higher transcript amount in the roots of plants of transformed line NKS-30 was related to faster

root growth and development of an extensive root system. Overall, this study has provided evidence that expression of cyclin coding sequences in transformed banana is related to growth promotion. Specifically, *Arath;CyclinD2;1* promoted shoot growth while the *Musa* homolog promoted root growth. Shoot and root growth phenotypes obtained in this study might have the potential to improve banana productivity in terms of short plant growth cycle, increased bunch weight, improved plant anchorage and increased plant resistance to root nematode damage. Future work should assess the produced plants in the field to allow transformed plants to exhibit their full potential and to be able to fully evaluate the vegetative and flowering phases.

Thesis composition

Chapter 1 describes the botany of the banana plants and the demand for a fast maturing banana plant and extensive root growth and the possible contribution of these characteristics to productivity. This chapter also contains the limitations of conventional methods to breeding for these traits and the molecular option targeting the *CyclinD2*-type gene to accelerate the cell cycle. *Chapter 2* covers the results obtained for isolation and characterization of the *CyclinD2;1*-type gene ortholog from banana including a phylogenetic analysis of the isolated banana cyclin gene sequence. *Chapter 3* describes the creation of Arabidopsis and banana *CyclinD2;1* gene constructs suitable for banana transformation, their delivery into transformation into banana cells using the Agrobacterium-based transformation system, regeneration of transformed plants and finally the detection of the transferred transgene in transformed banana using molecular biology tools. *Chapter 4* reports about the expression analysis of both the endogenous cyclin banana gene and of transferred cyclin transgenes in selected regenerated banana plants using the technique of quantitative RT-PCR. *Chapter 5* outlines the phenotypic evaluation of transformed banana plants to detect any changed shoot and root growth characteristics and to determine a possible relationship between changed characteristics and transcript amounts of transgenes. Finally, *chapter 6* summarizes the novel results found in this study and evaluates the set initial working hypothesis. Further, recommendations for further research work, based on the results obtained in this study, are outlined.

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