CHAPTER SIX

GENERAL CONCLUSION AND RECOMMENDATIONS

In general, studies with transformed banana have the disadvantage that transformed shoots represent the final product of a transformed plant. Banana plants are triploids and predominantly sterile disallowing any self-fertilization and therefore the resultant phenotypes are fixed. In particular, somaclonal variation, caused by the tissue culture process, cannot be excluded which might result in phenotypic changes that are not related to the expression of a transgene (Filipecki and Malepszy, 2006).

A first new aspect of this study was the successful isolation of a *CyclinD2;1* gene coding sequence from banana. This addressed the objective set to isolate a cell cycle (*CyclinD*-type) gene homologue from banana and to determine the level of homology of banana cyclins with those of other plant species. Phylogenic analysis provided strong evidence that the banana cyclin is more related to known monocot cyclinD-types than to the Arabidopsis homolog. This phylogenic grouping was based on the overall amino acid sequence which is reported to be less conserved in cyclins (Vandepoele *et al*., 2002; Menges *et al*., 2007). Further, functional homology between the Arabidopsis and banana *CyclinD* was also possibly sufficient to cause interactive effects of the two orthologs in transformed plants. Particularly in roots, high *Arath;CyclinD2;1* transcription resulted in much lowered transcription of the endogenous banana *CyclinD2;1*.

In a second new aspect, transformed banana were produced over-expressing either the Arabidopsis or the banana *CyclinD* coding sequence to allow phenotypic evaluation. This addressed the set objectives to over-express *Arath;CycD2;1* and *Musac;CycD2;1* in banana and to evaluate the phenotypic effect on the expressed *CyclinD2;1* on the growth and development of banana plants. An interesting observation was that *CyclinD2;1* transformed banana plants had a higher regeneration and transformation rate compared to cells only co-cultured with an empty vector pBin19. Transformation and regeneration competence is reported to be high in cells when transformed while in the actively dividing stage (Arias *et al*., 2006). The banana *CyclinD2;1* was isolated from an actively proliferating cell suspension and was also confined to the banana meristematic shoot and fruit tissue linking it to the cell cycle. *CyclinD* is associated with cell proliferation where it plays a regulatory role at the G1/S transition phase of the cell cycle (Dewitte and Murray, 2003; Inzé and De Veylder, 2006). A future study might

confirm whether over-expression of *CyclinD* can indeed improve banana transformation and *in vitro* shoot regeneration.

A third new aspect of the study was the lack of any relation between various transcript amounts and phenotypic changes found in transformed plants. Plants of one line, D2-41, showed a high leaf elongation rate with an equally high *Arath;CyclinD2;1* transcription. In contrast, plants of line D2-12 with a similar leaf growth phenotype had the lowest transcript amount whereas plants of line D2-3, with an intermediary transcript amount of the *Arath;CycD2;1* and the least affected endogenous *cyclin* genes, showed the lowest leaf growth rate. Several factors may explain the lack of any direct relation between leaf growth phenotype and transcript amounts of a transgene. First, unlike growth measurements that were taken over time, transcription analysis was carried out by a one-time sampling. Thus, any difference in the pot environment could affect the cell cycle and therefore cyclin transcript amounts. Roots growth is reported to be more responsive to temperature and soil water potential (Walter *et al*., 2009). These environmental factors affect growth by restricting cell division through down regulating cyclin genes (Sacks *et al*., 1997; Rymen *et al*., 2007). Such differences can also partly explain the variability in transgene transcription exhibited by plants of the same line. Environmental influence on gene expression has been well-documented (Meyer, 1995; Down *et al*., 2001) and specifically a high response of *CyclinD2;1* transcription to cell cycle stimuli has been, for example, reported for Arabidopsis (Riou-Khamlichi *et al*., 2000; Dewitte and Murray, 2003). Such variability could have been reduced by mass propagation of plants of individual lines and pooling at least three plants as a biological sample instead of using only individual plants for analysis. However, such study would require extensive growth space to house a considerable number of plants with replicates, which is impossible to be carried out in the present growth facilities in Uganda.

A fourth new aspect was that only marginal enhanced leaf growth rates were found for transformed plants. This observation is probably attributed to gene redundancy that could have confirred resilience in the genome of the banana used in the study. Only one *CyclinD2;1* gene was overexpressed out of the large family of cyclins identified in Arabidopsis, maize and rice genomes (Menge *et al*., 2007; Guo *et al*.., 2007; Hu *et al*., 2010). Thus, unlike in tobacco where overexpression of Arabidopsis *CyclinD2;1* gave

remarkable phenotype changes, upregulating only one gene member in a more complex plant like banana might have had limited effect on the growth phenotype. Another explanation could be that the glasshouse conditions under which the aerial growth was evaluated might have limited transformed plants from exhibiting their full growth potential. For example, a better growing root system in a transformed plant, as found in this study for transformed plants, can quickly outgrow the pot volume limiting the growth rate of such plant. Dosselaere *et al.* (2003) observed restricted root growth and development of potted banana plantlets determined by pot size. During the present study, restricted growth was observed when attempts were made to grow banana plants in 50 L potting substrate to maturity. The plants grew bigger but both transformed and non-transformed plants flowered after two years compared to the nine months it would take in field conditions. It is known that final plant yield is determined by developmental and physiological processes, for which a single gene could play a major role (Van Camp, 2005). Therefore, to determine the effect of over-expression of cyclin on yield, a detailed evaluation of the produced transformed plants in a confined field trial will provide a more reliable assessment of the performance of the gene. Such studies will also consider the vegetative and reproductive aspects of the transformed plants. For example floral initiation in banana has been reported to be induced after a given number of leaves have been produced by a banana plant (Stover and Simmonds, 1997; Swennen and De Langhe, 1985). Thus, a plant with faster leaf growth, as found for plants of line D2-41, is likely to flower earlier. In addition, a positive correlation was reported in banana between the number of leaves produced and final bunch weight (Swennen and De Langhe, 1985). Both aspects should also be evaluated in more detail in future studies.

A fifth new aspect of the study showed that roots expressing the banana *CyclinD* exhibited faster *in vitro* root growth and the root system of potted plants of one line, NKS-30, was also visually longer. An extensive root system determines the plant's ability to obtain water and mineral nutrient (Taiz and Zeiger, 2006). The presented study provided first evidence that expression of *CyclinD* gene can be an interesting strategy to change root architecture and to obtain a better developed root system with longer roots. This could be a valuable trait for improving banana productivity in particular to improve drought tolerance. Blomme *et al* (2001) reported a positive

relationship between the banana root system and aerial plant growth. Breeding for extensive root systems was further suggested as one of the strategies to prevent nematode damage (Gowen, 1996). In addition, such root architecture can improve plant anchorage and can prevent plants from toppling under the weight of big bunches and during windy and wet seasons (Tenkouano *et al*., 1998). However, non-transformed plants of the same cultivar that were used to establish transformed plants should be tested if the root phenotype found in transformed plants also exists in a natural population. Therefore, more transformed and non-transformed banana lines should be produced in the future to evaluate in greater detail if *CyclinD* expression is a valuable strategy to improve banana rooting and improve performance against stressful conditions. Also, such studies should involve assessment if any effect of *CyclinD* on the root biomass also directly affects aerial parts.

At the formulation of this study, it was hypothesized that transformation of banana plants with a *CyclinD2;1* gene would accelerate the cell cycle that would result in accelerated banana plant growth. This study found some support for this original working hypothesis. In particular, expression of Arabidopsis *CyclinD2;1* caused faster leaf growth in one transformed banana line (D2-41) while the banana *CyclinD2;1* induced remarkable root growth in plants of line NKS-30. However, future evaluation of these transformed plants under natural growth conditions should be conducted to further support the hypothesis when plants exhibit their full potential and at the same time evaluate their vegetative and flowering phases.