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Functional analysis of the Arabidopsis ELO3 gene coding for histone acetyltransferase and component of the Elongator complex

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The Elongator core enzyme, ELO3, is a conserved histone acetyltransferase that probably functions in histone acetylation during RNAPII transcription elongation in plants. A role for Elongator in leaf and root growth was demonstrated. No report on flowering time, light regulated phenotype, and lateral root formation. Insight into the molecular mechanisms that direct leaf size, shape and leaf number will result in genetic tools to improve food and bio-energy production. Arabidopsis is a useful model system for studies of plant development. To investigate the genetic relationship between the *ELO3* and *ROT3* genes affecting leaf morphology, double mutant analysis was performed. Furthermore, *elo3* mutant phenotypes of leaf area, lateral root density, flowering time, and hypocotyl growth (short day, dark, far red, red and blue light) were studied in order to indentify functional role of the *ELO3* gene in plant development. Q-PCR was performed to determine the regulatory role of *ELO3* gene in light perception, circadian clock gene expression *(CCA1)* and phytochrome interacting factor3 *(PIF3)* expression. Fresh and dry weight was measured to determine the effect of the *ELO3* gene over expression on plant biomass. The results showed primary root growth, lateral root density and leaf area of *elo3* mutant were reduced compared to wild type. The *elo3-1* revealed early flowering phenotype in short day condition. Hypocotyl measurement of the *elo3-6* and wild type showed significant difference in short day condition and dark. The hypocotyl analysis of the *elo3* mutant in different light qualities indicated that there was also high significant difference in red and far red with $P<0.001$; however, no significant difference under blue light treatment was observed. The central circadian clock regulators *(CCA1, LHY)* were down-regulated in the *elo3* mutant compared to WT. The dry and fresh weight measurements of *ELO3* over expression line OE22 and WT had shown that the OE22 was higher in fresh and dry weight than WT. The *elo3 rot3* double mutant was identified from F3 progenies of *elo3-6xrot3-1* and the feature of the double mutant revealed the *ELO3* gene and *ROT3* genes act independently. The *ELO3* gene has functional role in controlling leaf morphology, flowering time, primary root growth, hypocotyl length, central circadian clock genes and a subset of light inducible genes expression more likely through its histone acetyltransferase activity with positive effect on plant growth and delays flowering in short day condition.

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Induction and evaluation of antitheilerial resistance in *Theileria* isolates

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Theileria parva is a tick borne disease causing East Coast fever (ECF) in cattle, a major constraint to livestock husbandry in eastern, southern and central Africa. Control of ECF is currently based on an infection and treatment method vaccine. Antitheilerial drugs, buparvaquone and halofuginone have been formulated targeting the parasite within the host cell. There is an urgent need to understand this parasite biology and develop alternative vaccines candidates for the future control of ECF. To explore the Biology of *Theileria* parasites; selectable markers are required for routine transfection which shall open avenues to understanding of the parasite and possibility of identification of resistance genes that could be used to generate candidate vaccine strains of the parasite in the future. Resistance to antitheilerial drugs is the first step to the achievement of resistance markers. *T. annulata* and *Eimeria* spp resistance to these compounds had previously been reported (Stephen *et al.,* 1997). Our study therefore focused on the use of antitheilerial drugs; buparvaquone and halofuginone to develop *Theileria* resistant cell lines and use molecular techniques to try and identify resistance markers using AFLP fingerprinting. We managed to develop *T. annulata* and *T. parva* resistant to halofuginone and buparvaquone through stepwise administration of each drug and achieved the highest concentrations of growth at 750 ng/ml in *T. annulata* and 40 ng/ml for *T. parva* in buparvaquone, however in halofuginone, we were able to develop resistance in *T. parva* up to a concentration of 60 ng/ml. Secondly, we performed AFLP on the two clones of parasite and the results generated were negative. Based on the results that were achieved, we recommend, the exploration of new purification techniques for isolation of schizonts and the further optimization of existing methods of purification, the incorporation of other genotyping approaches and restriction enzymes that favor the digestion of eukaryotic genome to improve the purification and AFLP profiles.
Key words: *Theileria* spp, schizonts, resistant markers, chemotherapy, AFLP fingerprinting

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Genome-wide analysis of genes involved in *Salmonella* Typhimurium biofilm formation by the Differential Fluorescence Induction (DFI) technique

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Salmonella Typhimurium is one of the most common causative agents of the food-borne diseases. The risk seems higher with the ability of bacteria to form biofilm on biotic as well as abiotic surfaces. However, studies conducted to get more insight into genetic regulations of biofilms have faced many difficulties due to the heterogeneity and environmental-regulated character of this process. In this study, we employed the Differential Fluorescence Induction (DFI) strategy, utilizing a FACS screening to isolate biofilm inducible promoters in *Salmonella* Typhimurium biofilms. The strategy was designed with alternative positive/negative sorting rounds to get rid of constitutive promoters as well as to enrich for biofilm induced promoters. The screening was performed using a *Salmonella* Typhimurium SL1344 promoter-probe library which contained around 20500 clones. 19 differentially expressed clones were isolated as they showed up-regulation of GFP expression in biofilm-induced conditions. Downstream analyses could identify 9 annotated promoters. Of these, *potF* and STM1851 were further analyzed by interrupting their sequences in the genome of two *Salmonella* Typhimurium strains, SL1344 and ATCC14028. *potF* encodes for the periplasmic-substrate binding protein of PotFGHI putrescine uptake system. Quantification of biofilm formation of mutants and complemented forms at 16^{o}C, 25^{o}C in 1/20TSB and 30^{o}C, 37^{o}C in CFA with and without addition of putrescine suggested a role for the transport machinery in the process. Mutants of two other polyamine transport systems (i.e *potA* from PotABCD, the spermidine uptake system and PotE, the putrescine transporter) in the bacteria were also constructed on the two strains and showed reduced biofilm forming capacity at 16^{o}C and 25^{o}C.
STM1851 encodes for a DUF1480 domain containing protein which is of unknown function. Biofilm quantification tests were carried out for STM1851 mutants at 16^{o}C and 25^{o}C in 1/20TSB. Drastic decrease in the level of biofilm formation was more pronounced in STM1851 mutants at 16°C and suggested a role of STM1851. However, further confirmation by complementation test is necessary. Preliminary results from

biofilm quantification tests of *potF*, *potA*, *potE* and STM1851 under different biofilm inducing conditions indicated possible involvements of these genes in *Salmonella* biofilm formation. In addition, the results also suggested different genetic regulations for biofilm formation in SL1344 and ATCC14028 and yet within one strain in different biofilm inducing conditions.

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Mechanism and modes of application of systemic RNAi in insects

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The **Desert Locust** (*Schistocerca gregaria*) is one of the most important agricultural pests in Africa, the Middle East and Asia due to its ability to form swarms which can fly rapidly for long distances (up to 200 kilometers in a day). Currently, chemical pesticides are intensively used to control the desert locust. Organophosphorous (OP) compounds, carbamates (CA), neonicotinoids etc. are being used as insecticides to control pest insects. OP compounds and CAs can inhibit the activity of acetylcholine esterase, whereas neonicotinoids can block the function of nicotinic acetylcholine receptors. These chemicals are neurotoxins and cause the death of the insect. Several very recent studies have shown that RNAi has the potential to control agricultural pests. Down regulation of the expression of specific genes through RNA interference (RNAi) has been widely used for genetic research in insects. In the present study cDNAs encoding a putative acetylcholine esterase (AChE) and a putative homologue of the α10-subunit of the nicotinic acetylcholine receptor have been studied to explore the effect of systemic RNAi, induced by exogenous injection of dsRNA into the hemocoel of the desert locust *S. gregaria*. All ESTs of the acetylcholine esterase (Contig 2863) and the nicotinic acetylcholine receptor (Contig1170) were resequenced to verify the 'consensus' sequences. As ESTs are only partially sequenced, additional sequencing reactions were performed to complete the sequence of Contig 1170. A T7 promoter sequence was attached at the 5' end of both sense and antisense primer and this primer pair was used in PCR reaction to generate a transcription template for the production of the dsRNA. 2.5 µg dsRNA of Contig 2863 and Contig 1170 was used for injecting the experimental animal *S. gregaria*. Afterwards, the effect of RNAi was analyzed by Real-Time RT-PCR. Real-Time PCR showed a lower transcript level of Contig 1170 in the central nervous system of dsRNA-treated *S. gregaria* confirming that exogenous injection of dsRNA elicits a strong and systemic RNAi response. In addition, the expression of Contig 1170 transcript in different body parts of the desert locust was verified by Real Time RT-PCR. On the other hand, an apparent higher expression level of the Contig 2863 transcript was observed in brain and ventral nerve cord of experimental animals. To rule out a 'contamination effect' of dsRNA, leading to higher cDNA levels in samples from experimental animals, a new primer pair should be designed which is located outside the dsRNA region.

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Expression of *MUC13* and *MUC20* in relation to enterotoxigenic *E.Coli* sensitivity in pigs

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In the pig farming industry, diarrhea is a major problem. One well known form of diarrhea is associated with enterotoxigenic *Escherichia coli* (ETEC). ETEC originates from the faeces of piglets with ETEC diarrhea, asymptomic carrier piglets or sows. ETEC colonizes the small intestine by attaching itself to it with its fimbria, of which the F4 fimbria are the most important ones. Once ETEC is attached to the small intestine of the piglet, it can multiply itself and produce enterotoxins. These enterotoxins are responsible for the diarrhea in neonatal, suckling and post-weaning piglets.

Many efforts have been made to solve the problem of diarrhea in piglets through oral administrations of polyclonal antibodies, administering purified F4 fimbria, administering orally with beta-glucans and so forth. Furthermore, genetic research studies in pigs have been done to identify gene/genes responsible for susceptibility or resistance to $F4^+$ ETEC. *MUC* genes (*MUC4*, *MUC13* and *MUC20*) have been mapped on chromosomal region SSC13q41 which also harbors the F4ab/ac receptor for ETEC. This shows that these mucin genes are interesting positional candidate genes for the F4ab and F4ac receptors.

In this study, the *MUC* genes were amplified by using qRT-PCR using specific primers to verify their level of expression in small intestines between the piglets inoculated with ETEC and not inoculated and also between piglets suffering from diarrhea and those not suffering. The level of gene expression showed no significant difference between the groups compared.

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Mucosal delivery of protein antigens for the vaccination of poultry

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Chlamydiosis and Campylobacteriosis are two important zoonotic diseases. They cause major losses in poultry and human. Prevention of the disease is therefore required and vaccination seems to be a good strategy.
For the development of a vaccine, MOMP was taken as a vaccine candidate for *Chlamydophila psittaci* and FlaA was taken for *Campylobacter jejuni*.
As we are making protein based vaccine that is delivered mucosally, we want to check if binding of vaccine to mucosa improves the immune response. So, in order to increase the potency of the vaccine to bind to mucosa, we chose two types of adjuvants that were then linked to our antigen. They were OprI from *Pseudomonas aeroginosa* and FimH from *Escherichia coli*.
For Chlamydiosis, MOMP was transfected in COS-7 cells and identified by indirect immunofluorescence based on the use of monoclonal antibody 7B6III. The transfection was however not successful.
For Campylobacteriosis, the multisite gateway technology, a recombinant based cloning system, was used to fuse the antigen FlaA to the adjuvants. The entry clones *Fla*A in pDONRTM221 and *Opr*I in pDONRTM221 were prepared, the recombination of the genes to produce fusion proteins was not yet successful, for reasons that still have to be determined.
As the recombinant technique uses FlaA without signal peptides, so we wanted to compare two types of FlaA production, with and without signal peptide for periplasmic expression, and pET serves for expression with the signal peptide. Restriction cloning was performed for *Fla*A in pET-22b and the protein was expressed in *E. coli* BL21DE3*. No protein expression was found and the sequencing result showed a deletion in the *pel*B coding sequence. Probably the expression of FlaA with a signal sequence for periplasmic localization is somehow disadvantageous for the cells.

(*Key words: MOMP, FlaA, FimH, OprI*)

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Mechanism of action of Doc (Death on Curing) from Phage P1

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Toxin-antitoxin (TA) modules are diverse and present on plasmid and chromosome of almost all prokaryotes. They are composed of closely linked genes encoding a stable toxin that can harm the cell and relatively non-stable partner antitoxin, which protects the cell from the toxin life harming effect. Toxins, described so far, are known to interfere with vital cellular processes such as replication and translation targeting DNA gyrase and mRNA or ribosomes respectively. Antitoxin abrogates the poisoning effect of the toxin through non-covalent protein-protein contact forming a complex. Accidental release of the toxin from the complex lead to either cell death or growth arrest. Due to this fact the toxin is considered as a molecular time bomb. Even though the biological function of these modules is an ongoing debate, their molecular architect and properties are starting to get elucidated and it seems that these characteristics are conserved across all described TA systems.

Phenomenons of medical significance such as biofilm formation, bacterial persistence during antibiotic treatment, and bacterial pathogenesis have already been implicated to TA systems. TA systems owned by pathogens also becoming an attractive antibiotic target.

One of the several TA systems described so far include the *phd/doc* locus of bacteriophage P1 that represents the plasmidic form of addiction module. The *phd/doc* locus of bacteriophage P1 encodes the toxin Doc (Death on curing) and antitoxin Phd (Prevent host death). Antitoxin Phd has two distinct functions: it auto regulate transcription from its own operator and protect the cell from the toxin Doc. Site directed mutagenesis was employed to generate several selected Doc mutants believed to be associated with its functional activity, cloned into expression vector pET-21b, expressed and purified, and used for *in vitro* toxicity assay.

Bacteriophage P1 encoded Doc has previously been described as a protein that mediate efficient cell growth arrest and mimicked mechanism of action of the aminoglycoside antibiotic hygromycin B in which both targets 30s ribosomal subunit to inhibit translation and induce growth arrest. Consistent with this finding our *in vitro* Doc toxicity experiment demonstrated that Doc inhibit translation efficiently. Immediate (together with Doc) or later addition of Phd correspondingly neutralized and reversed Doc induced

in vitro translation inhibition. Furthermore, 1:1 complex formation between the partners was found to be enough for neutralization and reversal of Doc toxicity contrary to the already described heterotrimeric complex formation (P_2D) for Doc inactivation. Residue A61 and H66 on Doc found to be associated with functional activity of Doc.

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Optimisation and validation of Newcastle disease virus molecular diagnosis : detection by real time RT-PCR and molecular characterization by sequencing

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There has been a cascade of events in the molecular diagnosis of Newcastle disease virus. A widely used Real time PCR protocol based on the M gene of genome of NDV was developed by Wise and colleagues (2004) and has recently been under scrutiny by different laboratories because of the inadequacy in detecting a newly classified genome-class I PMV1 isolates as characterized by Czegledi and colleagues (2006). Sequel to this ,L. Kim and colleagues(2008) developed an assay based on the L gene and tested in multiplex with the M gene based protocol, being able to detect both class II and class I isolates.
We validated the use of the multiplex assay and compared the result to the M gene assay. We evaluated the multiplex assay using four parameters; Analytical specificity, Analytical sensitivity, Diagnostic specificity and sensitivity and repeatability.
Our analytical specificity results shows that the test was able to detect all APMV1 strains, representing a diversity of genotypes, the analytical sensitivity (detection limit) reveals that the multiplex test was able to detect 10 and 15 viral copies of PMV1/pi/Belgium/1.3 and Ulster/ck/64 (Class II PMV1 isolates) respectively. While being able to detect 160 viral copies of PMV1/Germany/R49/99(Class I isolate).
In order to evaluate the diagnostic specificity and sensitivity, we compared the multiplex test to the M gene singleplex assay and we used Cohen's Kappa coefficient (k) to interprete the agreement between the two tests. The k value was calculated to be 0.75 implying that there is a substantial agreement between the two tests. Relative sensitivity and specificity compared to the singleplex M gene assay were 79.16 % and 93.80 %, respectively.
The coefficient of variation between runs (interassay variability) ranges from 1.812% to 4.75% depending on the virus titer in the reference sample. As expected, this is higher than the coefficient of variation within runs (intra assay variability) which ranges between 0.46% and 1.81%. The two results obtained (interassay variability and intra assay variability) shows a good repeatability of the multiplex assay.
Using random hexamer priming for cDNA production, we optimized our NDV sequencing protocol and obtained a 70% perfomance rate on our primers designed to amplify the genome of NDV. Molecular Diagnosis of NDV 11 Specific primers were also

used to produce cDNA and these yielded more results in amplification. The full length of the F gene of twelve field isolates from Nigeria were also sequenced. Phylogenic analysis of five of these isolates reveals 3 different lineages of velogenic NDV. Two different fusion gene cleavage sites were identified, both being considered molecular markers for velogenic ND viruses.

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Molecular characterization of Trypanosomes causing Equine Trypanosomosis in Arsi-Bale highlands of Ethiopia

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Equine Trypanosomosis is caused by different Trypanosoma species of which T. evansi and T. equiperdum are the cause of surra and dourine, respectively. Despite the difference in names of the two diseases and mode of transmission, the taxonomic classification of the causative parasites is under question. In this study conducted on samples collected from two dourine hot spot districts of Arsi-Bale highlands of Ethiopia; preliminary epidemiological factors such as the clinical forms of dourine according to the farmers, case prevalence study by Woo test, RoTat 1.2 and 18S PCR and serological study and finally characterization of two isolates by Random Amplified Polymorphic DNA were conducted. The epidemiological study revealed that nervous, genital, emaciation and combination of these are the major clinical forms described. Furthermore, for the first time the parasite was demonstrated by Woo test in 4.6% of the horses, and RoTat 1.2 and 18S PCR recorded a case prevalence of 36.7% and 47.6% while 27.6% of the horses demonstrated antibodies against the trypanosome by CATT/T. evansi. The result indicated that equine trypanosomosis is highly prevalent in the area and more interestingly, the animals are positive for the tests developed specifically for diagnosis of T. evansi infection suggesting the disease is caused by T. evansi. Further, the study showed no significant difference in case prevalence of the disease between districts, sex and age groups of the animals. However significantly higher ($P > 0.05$) prevalence was recorded in emaciated animals as compared to animals of good body condition. Additionally optimization of a diagnostic real time PCR was carried out using RoTat 1.2 PCR that would give an alternative option for the diagnosis of the disease. Remarkably, molecular characterization by Random Amplified Polymorphic DNA shows that the two Dodola strains tested have a DNA finger print similar to T. equiperdum OVI rather than a classical T. evansi pattern. In general, the study therefore revealed that the cause of equine trypanosomosis recognized as dourine by farmers based on its clinical signs in Arsi-Bale highlands of Ethiopia seems to be T.(b) equiperdum. For confirmation of this finding sexual transmission study and genome sequencing is recommended .

Key words: CATT/T. evansi, Ethiopia, 18S PCR, RADP, RoTat1.2 PCR, T. equiperdum, T. evansi, Woo test.

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Development of monoclonal antibody against Aminopetidase N (pAPN) and investigating the binding of F4ab/ad enterotoxigenic *Escherichia coli* to pAPN and Porcine Brush Border Membrane Vesicles

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F4 ETEC causes diarrhea in pig and resulting in important economical loss. Expression of different F4 receptors on the pig intestine influences in their susceptibility for this pathogen. APN is one of the F4 receptors. To study the interaction between F4 ETEC and pAPN, mAbs against pAPN have been developed and the binding reactions between F4 ETEC and pAPN as well as BBMV F4R$^+$ were performed. In the first part of this thesis, 20 BALB/c mice were immunized with either pure pAPN or BHK-pAPN using 3 different immunization strategies (conventional immunization, neonatal tolerisation, and adult tolerisation) associated with 3 different adjuvants (Freund's, Montanide^{TM} ISA206, and ISA50) and were sacrificed for producing hybridoma. Five out of 8 neonatal mice died several days after tolerisation while 2 out of 6 mice following adult tolerisation died after treatment with Cy and Freund's adjuvant injection. Mice following conventional strategy were alive and gave highest responses when injected with Freund's adjuvant. Three fusions associated with 3 of those mice gave a total of 3575 wells containing hybridoma (110, 1557, and 1908 for mouse 1, 2, and 3, respectively) in which 93 wells were positive against pAPN when screened by ELISA (6, 84, and 3 from mouse 1, 2 and 3, respectively). Only 12 clones were stably producing antibodies against pAPN after expansion. Six out of these 12 clones were negative against pAPN when checked by ELISA but all of them showed binding to either pAPN, or BBMV F4R$^+$ or BHK-pAPN on the blots at different bands. Four groups of clones could be differentiated based on these results. In parallel, 12 stable hybridomas were cloned by limited dilution and gave 81 single-cluster containing wells. From those, 10 out of 54 tested wells were positive against pAPN in ELISA. They were cloned once more to make sure to have monoclonal populations. Clone 2.6C4 was the most promising. In the second part, F4ab/ac/ad fimbriae were allowed to bind pAPN on blots. Then the sugar-dependent binding activity was characterized by $NaIO_4$ cleavage reaction combined with immunoblotting. F4ab bound to β and γ fragments, F4ac bound to α and β fragments while F4ad bound to all 3. It was found that the binding of F4ac and F4ad to APN is probably dependent on the sugar moiety while the binding of F4ab is not. Although there were several potential dots on 2-D gel blots after immunoblotting of BBMV with F4ab/ac/ad, there is not enough evidence to confirm that those are APN and that APN is receptor of F4ab/ad due to bad negative control. Further evidence can be obtained using the newly produced mAbs.

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Evaluation of the water use efficiency of different *Musa* varieties : development of a sorbitol induced osmotic stress *in vitro* model

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The identification of drought tolerant *Musa* varieties normally takes place in the natural environment. However this process is time consuming and complicated by difficulties in field management, variation in phenotype and unexpected rainfall events. A technique that shortens the screening process would be of great importance. The general objective of this study was (i) to develop an alternative and rapid technique to screen the drought tolerant *Musa* varieties by an *in vitro* model and (ii) to get a first insight into drought tolerance via this model.

In search of a good model to screen, the effects of sucrose and sorbitol on growth of *Musa* species were compared. The results of this experiment proved that sorbitol is not a source of energy in the *in vitro* culture of *Musa* species and could be used in a model as a neutral osmotic inducer. The exploration of different levels of water stress induced by 0.1 to 0.5 M sorbitol in the media and their effects on the growth of *Musa* plants proved that the concentration of 0.2 M sorbitol is the ideal concentration to reveal different growth characteristics. The application of this concentration on a broad range of *Musa* cultivars (Mbwazirume (AAAh), Williams (AAA), Popoulou (AAB), Cachaco (ABB), Obino l'Ewai (AABp) and Lep Chang Kut (BBB) showed that all cultivars are affected by the sorbitol induced osmotic stress but the degree of sensitivity is different. Cachaco and Williams revealed a higher growth rate whereas the growth rate of Mbwazirume and Lep Chang Kut was very low under stress conditions. Lep Chang Kut does not perform well under *in vitro* conditions. The determination of the growth reduction due to sorbitol osmotic stress proved that the reduction of gain of dry weight was 5.56, 14.29, 20.00 and 25.00 % respectively for Cachaco, Lep Chang Kut, Williams and Mbwazirume. This study pointed out that the gain of fresh and dry weights of leafs; gain of leaf area and total gain of fresh and dry weights seem to be appropriate growth parameters to identify drought tolerant *Musa* cultivars under *in vitro* condition. The results of proteomics analysis showed a separation of different proteins but due to time constraints it was not possible to get a first insight into the drought tolerance of *in vitro* plantlets.

Through this study, an *in vitro* technique to screen drought tolerant *Musa* cultivars was developed and the drought tolerance of Cachaco and and Lep Chang Kut and drought sensitivity of Mbwazirume were proved.