https://dx.doi.org/10.4314/rj.v2i1.2D

Riboflavin induced tolerance against the root-knot nematode *Melopidogyne graminicola* in rice.

Nsengimana Joseph[a*], Ateka Elijah Miinda [b], Nsabimana Antoine[a] and Nyende Aggrey Bernard[c]

[a] Department of Biology, School of Science, University of Rwanda, P.O. Box 3900 Kigali, Rwanda.

[b] Department of Horticulture**,** Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, City Square, Nairobi, 00200 Kenya, eateka@agri.jkuat.ac.ke, Tel: +254 067 5271.

[c] Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, City Square, Nairobi, 00200 Kenya**,** abnyende@agri.jkuat.ac.ke, Tel: +254 077 52711/52181.

*Corresponding author: josephnsenga01@gmail.com, Tel: +250 785122932

ABSTRACT

The role of plant hormones such as Salicylic acid, Jasmonic acid and Ethylene in plant defense has been largely investigated particularly in dicotyledonous plants. Riboflavin has also shown effects on plant physiology but its role in plant defense/tolerance is yet to be elucidated.

Rice plantlets were randomly grown in laboratory, sprayed with hormones and riboflavin 24 hours before their infection by the nematodes *meloidogyne gramicola* juvenile 2. Two types of infection experiments were done. The normal rice nipponbare susceptible to *M. gramincola* infection was used in the first while in the second series experiments four transgenics/mutants were used. The shoots length, roots length and the roots weight were measured. The numbers of galls per plant were counted under a stereomicroscope. The relative expression of the genes *Osein2b, Oswrky 45, OsJAmyb* and *OsNPR1* was evaluated using the qRT-PCR method.

It was noticed that the plantlets treated with BTH significantly developed less galls compared to control ($P = 0.000<0.05$). This was also observed with ETHEPHON and MeJA spray ($P =$

0.001 and P = 0.000 <0.005 respectively). The treatment with riboflavin didn't show a significant effect (P = 0.61>0.05) in comparison with the control. However these plants grew taller enough with a strong roots system except for OsNPR1antisense line which developed more galls than others tested plants.

The foliar supply of riboflavin on rice significantly upregulated *OsNPR1* in leaves and roots of rice plantlets. Os*WRKY45* was also upregulated in both leaves and root samples.

Key words: Riboflavin, systemic tolerance, transgenics/mutants, gene expression

1. INTRODUCTION

Plant hormones play a vital role in plant survival and are involved in regulating plant growth, development and reproduction (Robert-Seilaniantz *et al*., 2011, Georgina D. Arthur *et al*., 2007). The role of plant hormones in plant defense has been investigated mostly in dicots like Arabidopsis (Balzan, S *et al*., 2014), but still a lot needs to be done in monocots (De Vleesschauwe, D *et al*., 2014). The dicotyledonous plant model *Arabidopsis thaliana* was intensely used to elucidate the production and interrelated role of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) in plant defense following pathogen attack.

It is known that rice is a staple diet in 39 countries and its demand is increasingly high as the world population keeps increasing (FOA, 1984). High yield in rice farming is compromised by many diseases and pests like the root-knot nematodes (RKNs). The problems caused by these nematodes in rice may increase in the near future as people are looking for growing rice in upland where they cultivate the so-called aerobic rice (Mew *et al*., 2004, Peng *et al*., 2006). It was noticed that *M. graminicola* is the most damaging nematode in aerobic rice fields. In their report Kamrun et al (2011) stated that there is a strong need of information from fundamental research in order to get more insight on the interaction between these nematodes

and the rice system. They presented an in-depth characterization of the role of SA, JA and ET in systemic protection of the rice against *M. graminicola*.

Moreover it is known that riboflavin (or vitamin B6) is produced by both animals and plants and, like most of other vitamins it acts as a coenzyme in the physiology of animals, plants and microorganisms (Dong, H and Beer, S.V., 2000). It has been demonstrated that this vitamin is involved in the process of antioxidation and peroxidation processes known to play a role in oxidative burst leading to hypersensitivity reactions (Jabs *et al*., 1996). Riboflavin induced the transcription of *PR* genes in arabidopsis and tobacco which triggered the systemic resistance of this plant against *Peronospora parasitica* and *Pseudomonassyringae pv.tomato*. In tobacco and tomato the action of riboflavin protected the plants against *Tobacco mosaic virus* (TMV) and *Alternaria alternata* (Dong, H and Beer S.V., 2000).

Furthermore it was proven that the use of protein kinase inhibitor K252a cancelled the effect of riboflavin in tobacco and arabidopsis. This phenomenon was also observed when riboflavin was sprayed on this plant mutated in the NIM1/NPR1 gene. However, the spray of NahG plant with riboflavin solution (10μM) induced the same resistance as in normal plants. In light of these observations Dong, H and Beer, S.V., (2000) concluded that the foliar spray of riboflavin elicits systemic resistance in *arabidopsis thaliana* and *tobacco* and activates novel signaling process in the plant and, went further to say that riboflavin-induced signaling requires functional protein kinase which is regulated by NIM1/NPR1.

In this research, the rice variety nipponbare known to be susceptible to infection by nematodes was used to investigate the protective capacity of exogenous supply of riboflavin against the nematodes *M.graminicola*. To get further insight on possible connection as well as cross-talking between riboflavin, SA, JA and ET pathways in rice-nematodes interaction, another infection experiments on transgenics/ mutants rice was done. Finally the quantitative

real time, qRT-PCR was used for analysis of gene expression following the treatment of rice seedlings by riboflavin.

2. MATERIAL AND METHODS

2.1 Infection materials: Two types of infection experiments were done. In the first experiments, the normal rice nipponbare was used. The rice (*Oryza sativa*) cultivar, Nipponbare is known to be susceptible to *M. gramincola* infection. The control was the cultivar Nipponbare rice sprayed with water/Tween. In the second set of experiments four transgenics/mutants rice were used. These are (1) Ein2b-antisense transgenic line. This is a transgenic rice with a silenced ein2b gene. The ein2b functional gene is a central component of ethylene signaling (Bailey *et al*., 2009); (2) Hebiba mutant. This is a Jasmonic acid pathway mutant. Hebiba was found to be defective in the gene encoding allene oxide cyclase (OsAOC) (Riemann *et al*., 2003); (3) NahG transgenic rice is a transgenic rice that expresses the nahG gene from a bacterium to produce salicylate hydroxylase that degrades SA into cathecol (Yang *et al*., 2004); (4) OsNPR1 antisense line. This is a transgenic rice whose OsNPR1 functional gene version controls the transcription of plant defense gene and it is responsive to Riboflavin (Dong, H and Beer, S.V 2000); (5) Nihonmasari rice was used as a control for Hebiba rice.

Infective organism: The *M. graminicola* culture which was originally isolated in the Philippines was maintained in vivo on wild type Nipponbare plants and grasses (*Echinocloa crus-galli*) in green house. It was used as the infective organism.

Plant hormones, riboflavin and their preparations: These plant hormones were prepared in water containing 0.02% (v/v) Tween 20. Ethephon, Ethylene releaser was prepared as (500 μM); MeJA (500 μM); BTH, salicylic acid analog (250 μM pre-dissolved in some drops of ethanol); and riboflavin (10μM) in water. Along with the preparation of hormone solutions, 2

other solutions were prepared to spray the control plants (C). C1 (Water and Tween) and C2 (Water + ethanol + 10 µl tween).

Substrate for rice culture and its infection by Nematodes: For nematode infection experiments, the SAP-substrate (Reversat *et al*., 1999) was used. PVC (polymer, polyvinylchloride) tubes were filled with SAP-substrates, rice plantlets were allowed to grow on and hoagland solution was added to the seedlings as source of nutrients.

2.2 Infection experiments: The rice seeds were geminated on a Petri dish (θ= 14 cm) on a wetted tissue paper. The seeds were incubated at 30°C for 4 days in darkness, then moved to light for 2-3 days. PVC-tubes were filled with SAP-substrates. The rice seedlings were transplanted onto SAP at the 7^{th} days from germination set up. The rice seedlings were sprayed with hormones at their 12^{th} days. These rice plantlets were infected with nematodes 48 hours after hormones sprays. Plantlets were kept in controlled rooms for 15 days after their infection. Data were then collected in this way: each plantlet was pulled out of the tube; roots were thoroughly washed with tap water to remove all the SAP. The shoots length as well as roots length were measured. Also the number of galls formed on roots was counted after staining with fuchsin as follows: the roots of each plant were tightly wrapped in a piece of well labeled mira cloth and submerged in boiling fuchsin acid staining dye (75 mg acid fuchsin, 50 ml lactate, 50 ml glycerol and 50 ml distilled water) for 2 to 3 minutes. The roots were washed with tap water, unpacked from the mira cloth, placed in 16 well-plates (5ml of distaining solution, 1:1 glycerol and distilled water with some drops of lactic acid in each well) and distained for at least two days on a shaker. The number of galls developed per plant was counted under a stereomicroscope. Collected parameters viz shoots length (sl), root length (rl), root weight (rw), number of galls per plant (gl) were analyzed using SPSS 16.

Data were first analyzed for normality (P>0.05) then data fitting for this quality were further analyzed using the one way ANOVA; P<0.005) and treatment were compared by Tukeys Post Hoc test (P<0.005).

2.3 Relative expression of *Osein2b, Oswrky 45, OsJAmyb* and *OsNPR1* genes in rice following hormones and riboflavin foliar spray

For this experiment nipponbare variety was used. 12 days-old plantlets were sprayed with riboflavin solution until run off (more or less twelve sprayer shots). Both leave and root tissues were collected 24 hours after hormone spray. From a pool of twelve treated plants, two biological replicates were formed (6 plants each), and two samples were taken (roots labeled R, and leaves labeled L). The leaves were cut above the base, roots removed from the stem and immediately deep-frozen and stored in liquid nitrogen. The samples could later on be stored at- 80°C. Samples for the control plants (non-sprayed) were also taken collected.

2.3.1 Choice of both reference and target genes, Primer design

All primers were designed with the Primer3 program and are shown in table 1. Optimal product size was determined to 50-100bp, optimal primer size to 18-25bp, GC% to 40-60% and Tm (melting temperature) between 58-62°C. The amplicon was preferably as close as possible to the polyA tail to improve reverse transcription efficiency. Tm, self-complementarity and primer-dimerization were checked with Oligo Calc (Kibbe, W.A. 2007). Self dimerization and cross dimerization of each primer pair were checked again with Finnezymes Multiple primer analyzer (Finnezymes, 2010). Specificity was tested by blasting the primer sequences in NCBI. 4 target genes were chosen according to 4 important plants hormones pathways involved in rice defense. These genes are *WRKY45*, a SA-inducible gene.

JAmyb which is a gene responsive on jasmonic acid in rice; *OsNPR1*, gene inducible by riboflavin, *Osein2b*, an Ethylene-responsive gene. *eif 5c* was used as a control gene.

Table 1. Primers used in qRT-PCR.

Genes (Gene bank)	Primer	Sequence (5'→3')	Product size (bps)
osWRKY 45(NM_001061727.1)	WRKY 45F	GGACGCAGCAATCGTCCGGG	118
	WRKY 45R	CGGAAGTAGGCCTTTGGGTGC	
JAmyb(AY026332)	JAmybF	GAGGACCAGAGTGCAAAAGC	73
	JAmybR	CATGGCATCCTTGAACCTCT	
Osein2b(Os06g11069)	OseinF	TGACAGAGACGCCATACTCG	93
	OseinR	GTTTGCTCTTCTCGCACTCC	
Os NPR1 (NPR1061888.1)	OsNPR1F	CGAGGACAAGGAGGAGAATG	86
	OsNPR1R	ACGAGCAAACCACTGGAAAT	
EIF 5C, (LOC_Os11g21990.1)	EIF5C F	CACGTTACGGTGACACCTTTT	90
	EIF5C R	GACGCTCTCCTTCTTCCTCAG	
EXP (LOC_Os03g27010)	EXP F	TGTGAGCAGCTTCTCGTTTG	101
	EXP R	TGTTGTTGCCTGTGAGATCG	

In the table 1, the gene and its accession number, name of primer, sequence of the primer and the expected size of amplicon are respectively recorded from left to right

2.3.2 Total RNA extraction

The working place was treated with RNase ERASE (MP Biochemicals, Brussels, Belgium) to make the space RNase-free and only RNase-free materials were used. Plant tissues were ground vigorously in a mortar and pestle. The powdered tissues were collected in 1.5ml eppendorf tubes properly labeled (filled at half), 500μl TRI reagent (Sigma- Aldrich) were added and the tubes were vortexed, kept in a pot of ice and sonicated 3 times for 10 sec, then incubated for 30 min at room temperature (RT). 100µl chloroform was added on the tubes

that were flipped for 15sec by hand. The tubes were incubated for 5 min at RT, centrifuged for 15 min at 4°C. New, well labeled tubes were prepared. Always on ice, the upper layer in each tube was transferred to a new tube. Isopropanol was added in a quantity equal to 75% of the amount of the upper layer collected. The tubes were then vortexed and centrifuged for 30 min at 4°C. The supernatant was removed; the RNA pellets in each tube were washed with 400µl of RNase-free ethanol and tubes centrifuged for 5 min at 4°C. Ethanol was removed and the pellet was allowed to dry at room temperature for 1hour. The dried pellet was dissolved in RNase-free water (water containing the Diethylpyrocarbonate, DEPC) and the concentration read on NanoVue^{TM} spectrophotometer (GE Healthcare, 2010). The amount of RNA extracted was recorded

2.3.3 cDNA synthesis and cDNA quality checking

Prior to cDNA synthesis, the RNA concentration in samples were adjusted to have a concentration of 3.5 µg/14.2 µl template for cDNA synthesis. RNAs were first treated with DNaseI (Fermentas,), then by RNase inhibitor (1U/µl, GE Healthcare). The reaction medium was buffered using DNaseI + Mg (10X, Fermentas). The reaction tubes were incubated at 37°C for 30 minutes and EDTA (25mM) added. At this stage, the reaction mixture was 20 µl for each sample. 20 µl RNA was mixed with 2 µl dNTPs (10 mM, Invitrogen) and 1 µl oligodT (10 µM, Invitrogen), 4 µl RNAse-free water. This mixture was incubated at 65°C for 5 min. The tubes were put on ice. 8 µl 5X first strand buffer (5x, Invitrogen) and 4 µl DTT (0.1 M, Invitrogen) was added and the tubes were incubated at 42°C for 2 minutes. The tubes were put on ice. 1 µl SuperScript II Reverse Transcriptase (200 U/ µl), Invitrogen) was added and tubes were incubated at 42°C for 2hours. The total reaction volume was 40 µl. This volume was diluted to 100 µl and the cDNA quality checked. cDNAs were finally diluted 3 three time (to 300 µl total volume), before running the qPCR.

2.3.4 Quantitative real-time RT-PCR and post-run analysis

For each cDNA-sample, a mastermix (MM) with primers was prepared. The components of the MM were originating from the qPCR core kit "SYBR green I - NO ROX" (Eurogentec, 2015). 5µl of each cDNA sample was combined with 15µl of MM in a 72-Well Gene-Disc^{TM} using the CAS-1200^{TM} Automated PCR Set up robot (Corbett Life Science, 2009). All PCR samples were prepared in triplicates. A 15µl no-cDNA-MM was included as negative control.

The qRT-PCR reactions were performed in the Rotor-Gene 3000 machine (Corbett Life Science) and results were generated by the Rotor-Gene 6 software. PCRs program was as follow: 5' at 95°C and 40 cycles of 45 sec at 95°C, 45sec at 60°C and 30 sec at 72°C.

3. RESULTS AND DISCUSSION

3.1. Effect of hormones and riboflavin on rice infection by *M.graminicola*

In this research, it was noticed that there is a significant different ($P = 0.000<0.05$) between the control and the plants treated with BTH in terms of number of galls formed per plants. The spray of rice plantlets with BTH prior to their infection by *M.graminicola* protected the plants in way that few nematodes managed to establish themselves in the rice roots. This was also observed with ETHEPHON and MeJA spray ($P = 0.001$ and $P = 0.000 <0.005$ respectively). However the treatment with riboflavin didn't show a significant effect ($P = 0.61>0.05$) in comparison with control as illustrated in figure 1.

https://dx.doi.org/10.4314/rj.v2i1.2D

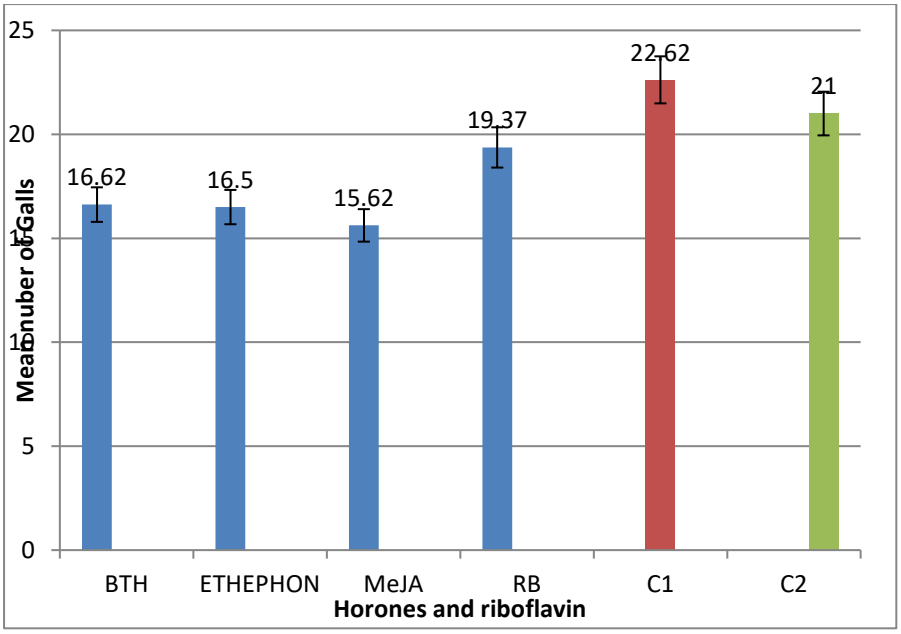

Fig 1. Results for infection of nippobare rice by *M.graminicola* 2 days post spray with 3 hormones and riboflavin.

Considering other agronomic parameters of tested rice, it was evident that riboflavin treated plants grew taller than other treatments and controls as exemplified by the shoots length comparison (Fig 2). It was clear that there is a significant difference in terms of shots length ($P = 0.008<0.05$) between the plant treated with riboflavin and the control.

The spray of riboflavin boosted the growth of rice plantlets in height in a significant way.

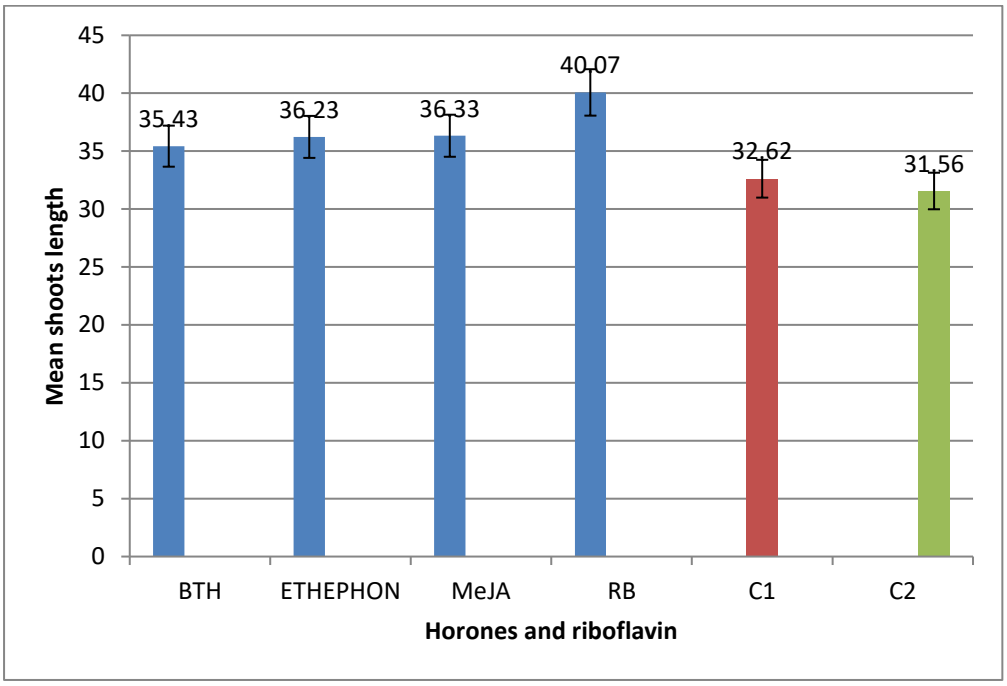

Fig 2. Comparison of shoot heights between three hormones treatment and riboflavin.

It was noticed that there were no differences between the controls and other hormones spray in terms of shoots length (P = 0.826 >0.05 for BTH, P = 0.348 >0.05 for ETHEPHON and P = 0.325 >0.05 for MeJA).

Moreover the rice plants treated with riboflavin developed stronger root systems (Fig. 3) where the RB-treated plants were 27% well rooted (in length) than the control.

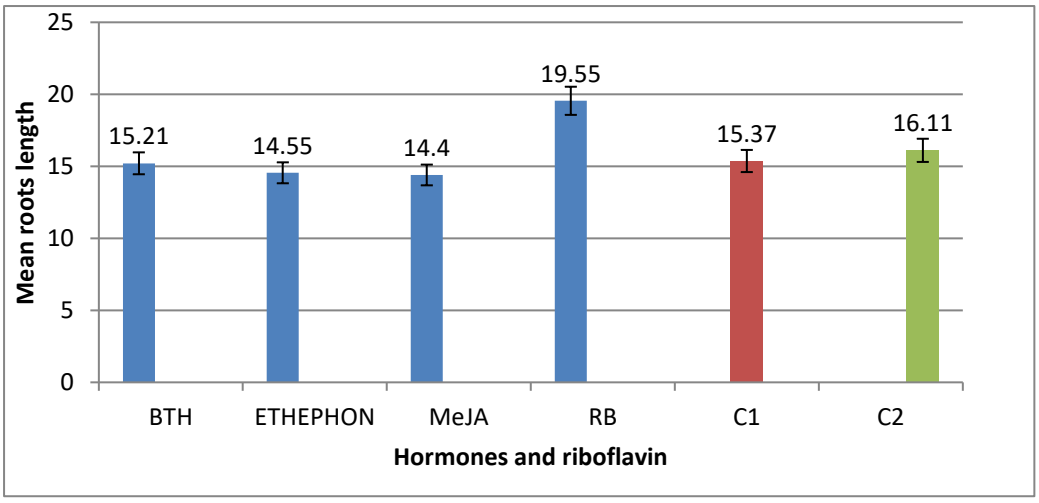

Fig.3. Results on the roots system development between plants treated with hormones and riboflavin.

In light of these results, it became clear that MeJA and ET sprays on rice seedlings confer a significant resistance in rice against *M. graminicola* infection. The same is true for BTH. Though the spray with riboflavin didn't significantly reduce the number of galls, the plants did not suffer from the infection by nematodes; it on the contrary grew taller and developed vigorous roots.

Other researchers demonstrated that riboflavin is involved in the process of antioxidation and peroxidation processes known to play a role in oxidative burst leading to hypersensive reactions. Dong, H and Beer, S.V (2000) found that Riboflavin induces the transcription of PR genes in arabidopsis and tobacco which trigger systemic resistance against *Peronospora parasitica* and *Pseudomonass syringae pv.tomato* in arabidopsis. In tobacco and tomato, riboflavin was proven to confer protection against *Tobacco mosaic virus* (TMV) and *Alternaria alternata,* respectively.

Kamrun et al. (2011) used the rice-nematode system to study in-depth the effect of plant hormones in rice protection and found that JA-biosynthesis and ethylene signaling are required for systemically induced defense against *M. graminicola.* This research with a focus on riboflavin effect on rice-nematode interaction found the same effects of JA/ET and revealed that plants treated with riboflavin continued to grow despite their infection with nematodes.

3.2 Effect of riboflavin on inducible genes and the pathway of plant hormones

3.2.1 Effect of riboflavin on transgenic/mutant rices

The results obtained from the infection tests of rice seedlings with impairment in one or the other pathway involved in hormones signaling pathway showed that plants sprayed with riboflavin solution prior to their infection by *M.graminicola* developed galls on their roots system. The assumption that the spray of riboflavin solution on transgenic/mutant rice plantlets produced the same infection effect as in normal, control plant rice", was made in designing the experiment. The analyzed results indicated that *M.graminicola* produced the same infection effect in both control

and treated plantlets. There is no significant difference in terms of galls formed between the treated plants and their respective control. P = 0.728>0.05 for hebiba compared with nihonimasari, P = 0.991>0.05, P = 0.888>0.05, P = 0.641>0.05 for ein2b antisense line, NahG and OsNPR1 mutant respectively compared to nipppnbare control.

However though not statistically significant it can be noticed that hebiba and OsNPR1 have developed more galls than their respective control with 12.85% and 12.26 % respectively (Fig.4).

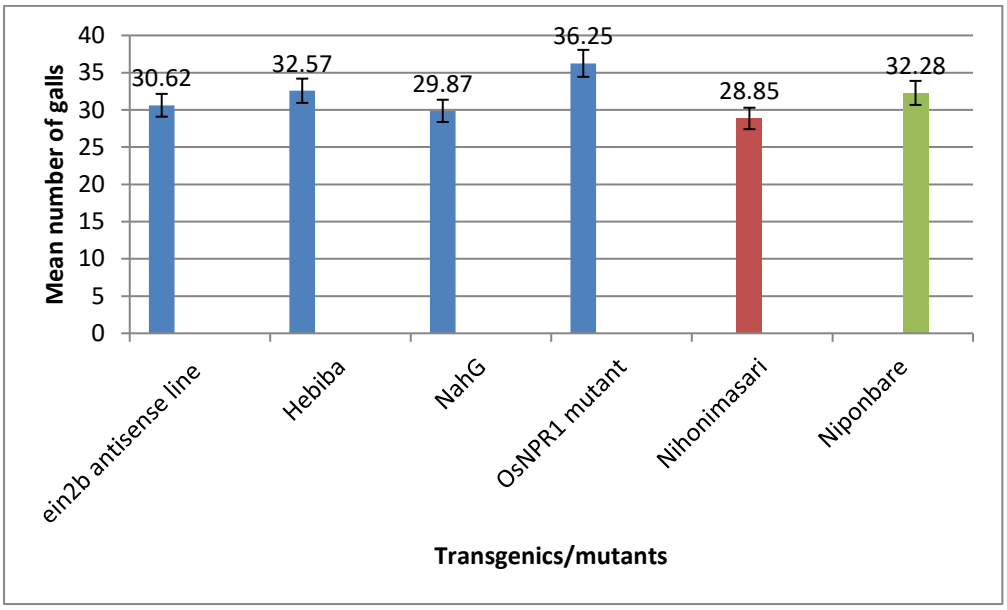

Fig 4. Results for the spray of riboflavin on transgenics/ mutants.

The comparison between MeJA and riboflavin showed that mutants in OsNPR1 treated with riboflavin suffered from nematode infection and had their shoots length reduced compared to the nipponbare control while MeJA protected the mutant.

It was documented that defense signaling pathways in plants work through a complex network of synergistic and antagonistic interactions (Koornneef and Pieterse, 2008).

Kamrun et al. (2011) used the same system of transgenics/mutants to study the effect of SA, JA and ET and found that even if there is a correlation between hormone pathways, especially JA/ET; it is the JA pathway that plays a major role in JA/ET-mediated protection of rice. In this work the same

rice-nematodes system and trangenics/mutants were used. And it was noticed that riboflavin uses a different pathway to mediate the rice tolerance toward *M. graminicola*.

3.2.2 Effect of riboflavin on pathways of targeted plant hormones

The quantification of RNAs from both leaves and roots of rice plants was done first in this series of experiments. The results (table 2) showed that the amount of RNAs (in µg/ml) obtained from leave samples was largely high than the amount of RNAs from roots where for instance COL1/COR1 was more than 3 folds, RBL1/RBR1was 2.4 folds**.**

Table 2. Amount of extracted RNA from Leaves and root tissues

Samples	**Concentrations (µg/ml)**
CO L1	1246
CO L2	1302
CO R1	391.2
CO R2	459.2
RB L1	1069
RB L2	1266
RB R1	437
RB R2	375

Total RNAs were extracted from both leaves and roots samples.

Reid et al., (2006) conducted a study on optimized grapevine RNAs isolation procedure and statistical determination of reference genes for RT-PCR during berry development. They extracted total RNA from grape pericarp, seeds, leaves, roots and flowers. They found that the yields were quite diverse depending on the source of the tissue. Leaves yielded the highest amount of total RNA (400–600 µg/gfw), followed by flowers, roots and pre-veraison seeds (150–300 µg/gfw), pre-veraison pericarp (40–120 µg/gfw), post-veraison pericarp (15–30 µg/gfw) and the lowest being

from post-veraison seeds (3–10 µg/gfw). The differences observed were likely related to the developmental and metabolic properties of each distinct tissue.

In this work more RNA quantities was obtained from the rice system compared to the quantities obtained from grape tissues (Reid, 2006). This suggests that the differences observed were not only related to developmental and metabolic properties of each distinct tissue but also the species of plants.

In order to check the quality of the synthesized cDNAs, two references genes were selected. These were Eukaryotic Initiation Factor 5C (EIF 5C) and Expressed protein (EXP).

As it is shown in the figure 5, the negative control was negative, and all the house-keeping genes have been amplified with their respective fragment sizes.

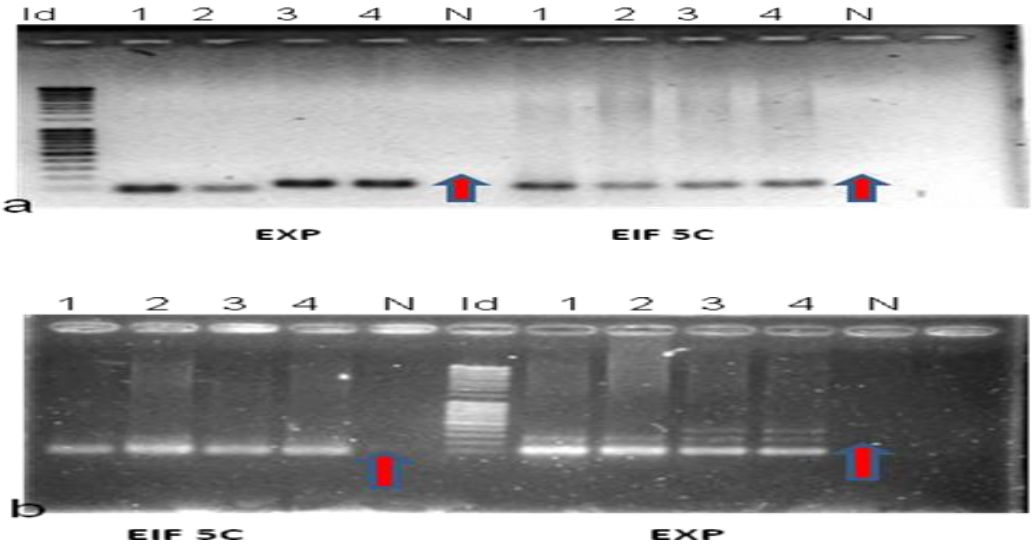

Fig 5. In picture (a) ld stands for ladder (or DNA marker) and in well 1, PCR products of the CO L1 leaves sample were loaded. In other wells samples were loaded as follows: in well 2 CO L2 leaves, well 3 RB L1 leaves and well 4 RBL2. The negative control lane is indicated by vertical arrow. In the picture (b), the same pattern was followed but with roots samples. The expected PCR product size was 101 for EXP and 90 for EIF5C.

EIF5C was retained to be used as reference gene in qRT-PCR. As it can be seen on both gel picture (a) and (b) of the figure 5, EIF5C bands were reproduced identically in both tissues, leaves (a) and roots (b).

Regarding the relative gene expression following the foliar spray of riboflavin solution, the results showed that Os*WRKY45* was upregulated in both leaf and root samples (Fig 6). The *OsNPR1* was significantly upregulated in both leaves and roots.

However, Osein2b and *Jamyb* responded negatively to exogenous supply of riboflavin with *Jamyb* being highly downregulated.

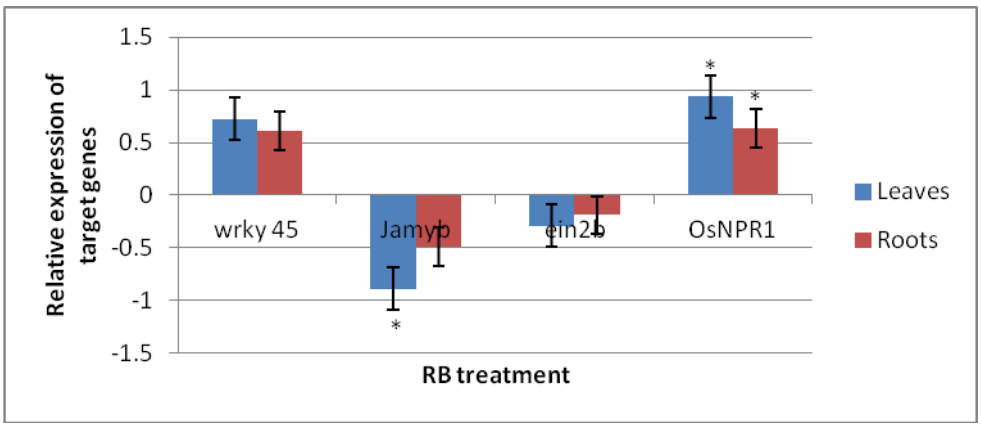

Fig 6. The relative expression of selected genes following the supply of exogenous riboflavin. On Y-axis are relative expressions in natural logarithms (lg_2). The control expression level was set to 1and $lg_2 1=0$. The start (*) indicates that the effect is significant compared with the control.

Jamyb was significantly downregulated in leaves and slightly in roots while Osein2b was slightly downregulated in both leaves and roots. As expected *OsNPR1* was significantly upregulated in both tissues following riboflavin treatment. The fact that *Oswrky45* was upregulated by riboflavin spray confirms the synergistic action between riboflavin and SA. This was observed on microbial pathogen on rice and it was proven on rice-nematodes interaction in this work.

The role of riboflavin as an elicitor of systemic resistance and a plant defense activator in rice as an important monocot plant was previously demonstrated by Paressa and Monica Höfte (2006) though they did it in rice-fungi system. They conducted study on the mechanism of riboflavin-induced-resistance and defenses response in rice against *Rhizoctonia* sheath diseases which are among the most important fungal diseases of rice, causing more than 50% yield losses in the world every year. These researchers found that riboflavin-IR can be linked to the induction of defense pathways leading to the formation of structural barriers such as lignin in rice plans. Their findings proved that using riboflavin as a plant defense activator can be a simple and environmentally safe strategy to control *Rhizoctonia* sheath diseases of rice.

4. Conclusion and recommendation

4.1 Conclusion

Contrary to the JA/ET and SA effect on rice-*M. graminicola* interaction where the hormones protected rice (as materialized by the reduced number of nematodes entering the roots), the treatment of rice by riboflavin didn't reduce the number of galls formed on rice seedlings. However, it was observed that the galls formed following riboflavin treatment were reduced in size compared to other treatments. The riboflavin-treated plants grew healthier; they were more exuberant than those sprayed with hormones despite their infection with *M. graminicola.* These strength and good health of the riboflavin-treated plants were proven by their long tillers and strong roots syetem. Knowing the involvement of riboflavin in oxidative burst, our results suggest that this riboflavin makes rice plants tolerant to *M. graminicola* infection by activating the reactive oxygen species arsenal which sequestrated the nematodes and prevented them to further growth.

4.2 Recommendation

More research works are needed to elucidate the interaction between riboflavin and other plant hormones like cytokinins and auxins as these hormones are involved in nematodes establishment in rice roots.

Acknowledgements

I am grateful to Ghent University, Belgium, where part of this work was done and the Belgium Technical Cooperation, BTC for financially supporting part of this work.

References

Bailey, T. A., Zhou, X. J., Chen, J.P and Yang, Y. (2009). Role of ethylene, abscisic acid and MAP kinase pathways in rice blast resistance. In Wang G, Valent B, eds, Advances in Genetics, Genomics and Control of Rice Blast Disease. Springer, Dordrecht, the Netherlands, pp 185–190.

Balzar, S., Johal, G. S and Carraro, N. (2014). The role of auxin transporters in monocots development. Front Plant Sci. 2014 5: 393.

Corbett Life Science. (2009). Rotor-Gene, a DNA amplification system; CAS-1200, a PCR set up robot; the CAS-4200 and CAS-4800 precision liquid handling workstations. Corbett Life Science Pty., Ltd.

De Vleesschauwer, D., Xu, J and Höfte, M. (2014). Making sense of hormone-mediated defense networking: from rice to ***Arabidopsis.*** Front Plant Sci.5: 611.

Georgina, D.A., Wendy, A.S., Ondřej, N., Petr, H., Johannes van, S. (2007). Occurrence of

nutrients and plant hormones (cytokinins and IAA) in the water fern Salvinia molesta during growth and composting. Environmental and Experimental Botany 61 (2007) 137–144.

Dong, H and Beer, S.V. (2000). Riboflavin Induces Disease Resistance in Plants by Activating a Novel Signal Transduction Pathway**.** The American Phytopathological SocietynVol. 90 No 8, 200.

Eurogentec. (2017). qPCR reagents and consumables. In http://secure.eurogentec.com/fields.

Finnezymes. (2010). Multiple primer analyzer. In http://themofisher.com/rw/en/home.

FOA. (1984). Rice consumption and nutrition problems in rice-consuming countries. In http:// http://www.fao.org/ag/portal/index_en/en/.

GE Healthcare, (2010). NanoVue Plus Spectrophotometer**.** In http://www.gelifesciences.com/webapp/wcs/stores/servlet/Home/en/GELifeSciences/

Jabs, T., Dietrich, R.A and Dangl, J.F. (1996). Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Science273:1853-1856.

Kamrun, N., Kyndt, T., De Vleesschauwer, D., Höfte, M and Gheysen, G. (2011). The jasmonate pathway is a key player in systemically induced defense against root-knot nematodes in rice. Plant physiology pp 111.177576.

Kibbe, W.A. (2007). OligoCalc: an online oligonucleotide properties calculator In http://biotools.nubic.northwestern.edu/OligoCalc.html.

Koornneef, A., Pieterse, C.M.J. (2008). Cross-talking in defense signaling. Plant Physiol 146: 839-844.

Mew, T. W., Leung, H., Savary, S., Cruz, C. M. V and Leach, J.E. (2004). Looking ahead in rice disease research and management. Crit. Rev. Plant Sci. 23: 103-127.

Parissa, T and Höfte, M. (2006). Riboflavin induces resistance in rice against Rhizoctonia

sheath diseases by activating signal transduction pathways leading to upregulation of rice cationic peroxidase and formation of lignin as a structural barrier. Comm. Appl. Biol. Sci, Ghent University, 71/1.

Peng, S. B., Bouman, B., Visperas, R.A., Castaneda, A., Nie, L.X and Park, H.K. (2006). Comparison between aerobic and flooded rice in the tropics: Agronomic performance in an eight-season experiment. Field Crop Res 96: 252-259.

Reid, K. E. , Olsson, N., Schlosser, J., Peng, F and Lund, S.T. (2006). An optimized grapevineRNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol. 14; 6:27.

Riemann, M., Muller, A., Korte, A., Furuya, M., Weiler, E.W and Nick, P. (2003) Impaired induction of the jasmonate pathway in the rice mutant hebiba. Plant Physiol 133: 1820-1830.

Reversat, G., Boyer, J., Sannier, C and Pando-Bahuon, A. (1999). Use of a mixture of sand and water-absorbent synthetic polymer as substrate for the xenic culturing of plant-parasitic nematodes in the laboratory. Nematology 1999;1:209-212.

Robert-Seilaniantz, A., Grant, M and Jones, J.D. (2011). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol. 49:317-43.

Yang, Y., Qi, M and Mei, C. (2004). Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. The plant Journal Volume 40.

https://dx.doi.org/10.4314/rj.v2i1.2D