

## **Evaluation of Nitrogen Use Efficiency (NUE) of rice (*O. sativa*) under saline conditions**

**Submitted by Mathieu Bonnavé**

Supervisor(s): Prof. Pierre Bertin (UCL/ELI/ELIA)  
Prof. Cuong Pham Van (VNUA)

Readers: Prof. Xavier Draye (UCL/ELIA)  
Prof. Stanley Lutts (UCL/ELI)

Thesis submitted for Master's degree in  
**Bioengineering: agronomic sciences**



## Acknowledgments

I would like to start by thanking my supervisor, Professor Pierre Bertin, for giving me the opportunity to realize my master thesis on this subject, for his great support and trust all along the year.

I would also like to thank my co-supervisor, Professor Cuong Pham Van, for giving me access to his facility for his welcome advice during my stay in Vietnam.

A special thanks to Nhung Phan, who was always available to answer my questions, for her help, her support over the whole year and especially during this experiment in Vietnam. Many thanks for her warm welcome and for facilitating my stay. It has been a pleasure to contribute on a small scale to her PhD thesis.

All my gratitude goes to Thomas Dagbert, Pierre Van Thorre, Claudine Givron, Anne Iserentant and Joseph Nader who made possible all the technical analysis of my experiments in Belgium with their expertise and assistance. Thanks to the Vietnamese students, Giang and Diu, who helped me nearly every day to manage and complete my experiment under the best conditions possible in Vietnam.

I would not forget to thank Tung, his family, Aurore, DaYan, Eugene, Pauline and Regina for their welcome, the discovery of Vietnam with them and all the time spend together.

Thanks to all my friends who made my studies in Louvain-la-Neuve and Pamplona a great time of my life.

I am very grateful to my family, and especially my parents for their support and their encouragement during all those years.

Finally, the stay in Vietnam realized as part of my experiments was made possible thanks to the financial support of the *Académie de recherche et d'enseignement supérieur* of the Wallonia-Brussels Federation, Belgium, in its development cooperation policy.



## Abstract

Rice (*Oryza sativa* L.) is a major food supply in the world and many challenges for its cultivation sustainability have to be faced. One of these challenges consists in an efficient management of the nitrogen fertilization. Indeed, nitrogen is one of the most important elements for the plant growth and development. However, rice cropping conditions can be favorable to nitrogen losses leading to problems like eutrophication. Developing varieties reaching higher nitrogen use efficiency could represent a valuable improvement for the crop yield and economically, but also a sustainable improvement. Another challenge is to deal with the increasing risk of abiotic stress due to climate change, such as salinity which is more frequently present in Vietnamese deltas.

This study consisted in an analysis of the interactions between three fixed parameters: the nitrogen fertilization applied, the salinity level and the rice genotype. Two first experiments were conducted in hydroponics during the early vegetative phase with two nitrogen concentrations (standard N and 0.125N) and three increasing salt levels (no salt, moderate and strong salinity) for three genotypes Cuom: Vietnamese salt tolerant; Pokkali: salt tolerant reference; Ngoi: Vietnamese salt sensitive). The study was completed with an experiment in pots in Vietnam to validate the findings of the hydroponics analysis. This last experiment was conducted until the harvest but was focused on the active tillering.

Several conclusions can be highlighted with this work. The first one was the absence of salt sensitivity before the seedling emergence. The second conclusion showed that the N toxicity seemed to increase with the salinity. Finally, the salt tolerance had an influence on the Nitrogen Use Efficiency (NUE). A high salt-tolerance cultivar, such as Cuom, presented a high absorption (aNUE) and agronomical NUE (agNUE) coupled to a low physiological one (pNUE), while the opposite NUE levels were more related to salt-sensitive cultivar. The Vietnamese in-situ experiment did not invalidate the conclusions but can only confirm the negative influence of salt stress on rice and the basic nitrogen effects in absence of salt. Nitrogen Use Efficiency is a valuable indicator to be considered in future studies regarding the salinity tolerance of rice.



## Abbreviations

ANOVA	Analysis of the Variance
CN ratio	Carbon-Nitrogen ratio
DF	Degree of Freedom
EC	Electrical Conductivity
ECAV	Ecophysiology and Plants Genetics for Sustainable Agriculture (ELI-A, UCL)
ELI-A	Earth and Life Institute - Agronomy (UCL)
GHG	Greenhouse Gases
GLM	Generalized Linear Model
GRPV	Plant physiology Research Group (ELI-A, UCL)
N	Nitrogen
IRRI	International Rice Research Institute
IRGA	Infra-Red Gas Analyzer
LAI	Leaf Area Index
LSMEANS	Least Squares MEANS
NUE	Nitrogen Use Efficiency
agNUE	Agronomical Nitrogen Use efficiency
pNUE	Physiological Nitrogen Use Efficiency
rNUE	Recovery Nitrogen Use Efficiency
QTL	Quantitative traits locus
Rubisco	Ribulose-1,5-biphosphate Carboxylase/Oxidase
SES	Standard Evaluation System
SPAD	Soil-Plant Development Analysis
SSE	Sum of Squared Errors
UCL	Université Catholique de Louvain
VNUA	Vietnam National University of Agriculture



# Table of contents

<b>Introduction.....</b>	<b>1</b>
<b>Chapter I: State of the art.....</b>	<b>3</b>
<b>1 Rice.....</b>	<b>3</b>
1.1 Generalities .....	3
1.2 Morphology of the Rice Plant.....	5
1.3 Physiological Phases of Rice .....	6
1.4 Rice Ecosystems.....	7
1.4.1. Generalities .....	7
1.4.2. Puddled Soils .....	9
1.5 The Future of Rice.....	10
1.5.1. Impact of Climate Change .....	10
1.5.2. Rice Response Options for the Future.....	11
<b>2 Nitrogen .....</b>	<b>12</b>
2.1 Nitrogen Cycle .....	12
2.2 Nitrogen Transformation in Flooded Rice Soils .....	13
2.3 Rice Nitrogen Uptake .....	14
2.4 Functions of the Nitrogen .....	15
2.5 Nitrogen Use Efficiency .....	16
2.6 Fertilizer N Management in Rice .....	17
2.7 Misuse of Nitrogen.....	18
<b>3 Salinity.....</b>	<b>18</b>
3.1 Effect of Salt Stress .....	19
3.2 Tolerance Mechanisms .....	21
<b>4 Interaction Between NUE and Salt Stress .....</b>	<b>21</b>
<b>Chapter II: Interest and Objectives .....</b>	<b>23</b>
<b>Chapter III: Materials and Methods .....</b>	<b>25</b>
<b>1 Part I: Evaluation of the Interactions between N, NaCl and Genotype in Hydroponics at UCL .....</b>	<b>25</b>
1.1 Material and Experimental Conditions.....	26
1.1.1. Plant Materials .....	26
1.1.2. Experimental Conditions .....	27
1.1.3. Treatments .....	27
1.2 Prospective Study at the Germination Stage.....	28
1.2.1. Experimental Specificities .....	28
1.2.2. Data Collection .....	28
1.3 Evaluation during Vegetative Stage .....	29

1.3.1.	Experimental Specificities .....	29
1.3.2.	Sampling.....	29
1.3.3.	Data Collection .....	30
1.4	Analyses of the Observations.....	31
<b>2</b>	<b><i>Part 2: Validation of the Interactions between N, NaCl and Genotypes in Pots at VNUA, North Vietnam .....</i></b>	<b>33</b>
2.1	Plant Materials .....	34
2.2	Experimental Conditions .....	34
2.2.1.	Fertilization.....	35
2.2.2.	Treatments .....	35
2.3	Sampling.....	36
2.3.1.	Data Collection .....	36
2.4	Analysis of the Observations .....	37
	<b><i>Chapter IV: Results.....</i></b>	<b>39</b>
<b>1</b>	<b><i>Experiments in Hydroponics .....</i></b>	<b>39</b>
1.1	Experiment during Germination.....	39
1.1.1.	Shoot Length.....	40
1.2	Experiment during the Vegetative Stage .....	41
1.2.1.	Morphological Adjustment .....	41
1.2.1.1.	SES .....	41
1.2.1.2.	Shoot Dry Weight.....	42
1.2.1.3.	Root Dry Weight.....	44
1.2.1.4.	Shoot/Root Dry Weight Ratio.....	44
1.2.1.5.	Number of tillers .....	45
1.2.1.6.	Shoot Length.....	45
1.2.1.7.	Root Length .....	47
1.2.2.	Physiological Adjustment.....	48
1.2.2.1.	Effects on Photosynthesis Function.....	49
1.2.2.1.1.	Assimilation Rate of CO <sub>2</sub> .....	49
1.2.2.1.2.	Transpiration Rate .....	49
1.2.2.1.3.	Stomatal Conductance .....	50
1.2.2.1.4.	Sub-Stomatal CO <sub>2</sub> Concentration.....	51
1.2.2.2.	Total Protein Content in Shoot.....	52
1.2.2.1.	Total Protein Content in Roots.....	53
1.2.2.2.	Chlorophyll Content.....	54
1.2.2.2.1.	α-Chlorophyll Content.....	54
1.2.2.2.2.	β-Chlorophyll Content.....	56
1.2.2.2.3.	Carotenoid Content.....	56

1.2.2.3. Nitrogen Content.....	56
1.2.2.1. Carbon Nitrogen Ratio.....	58
1.2.2.2. Nitrogen Use Efficiency .....	60
1.2.2.2.1. Absorption Nitrogen Use Efficiency .....	60
1.2.2.2.2. Physiological Nitrogen Use Efficiency.....	62
1.2.2.2.3. Agronomical Nitrogen Use Efficiency .....	63
1.2.2.3. Na Content.....	65
1.2.2.4. K Content.....	66
1.2.2.5. Sodium Potassium Ratio.....	68
1.2.2.6. Cl Content.....	69
<b>2 Experiment in Vietnam .....</b>	<b>70</b>
2.1 Morphological adjustment .....	70
2.1.1. Shoot Dry Weight.....	70
2.1.2. Root Dry Weight .....	72
2.1.3. Shoot/Root Dry Weight Ratio.....	72
2.1.4. Shoot Length.....	72
2.1.5. Number of tillers .....	73
2.2 Physiological Adjustment.....	75
2.2.1. Effects on the Photosynthesis Function .....	75
2.2.1.1. Assimilation Rate of CO <sub>2</sub> .....	75
2.2.1.2. Transpiration Rate .....	76
2.2.1.3. Stomatal Conductance .....	77
2.2.2. Agronomical Nitrogen Use Efficiency .....	77
<b>Chapter V: Discussion and Perspectives .....</b>	<b>79</b>
<b>3 Discussion .....</b>	<b>79</b>
3.1 Effects of the Genotype on Rice Salt Tolerance .....	79
3.2 Effects of Nitrogen on Rice Salt Tolerance .....	80
3.3 Characterization of Nitrogen Use Efficiency for Rice Salt Tolerance .....	81
3.4 Environmental Influences over the Experiments .....	82
<b>4 Perspectives .....</b>	<b>83</b>
<b>Chapter VI: Conclusions .....</b>	<b>85</b>
<b>Chapter VII: References.....</b>	<b>87</b>
<b>Chapter VIII: Annexes .....</b>	<b>93</b>
<b>1 Protocols .....</b>	<b>93</b>
<b>2 Script of the Statistic Analysis with SAS 9.4 .....</b>	<b>94</b>
<b>3 Additional Results .....</b>	<b>96</b>



## List of tables

Table 1: Rice varieties selected for the hydroponics experiments .....	26
Table 2: Nitrogen and salt concentrations applied as treatments in hydroponics.....	27
Table 3: Standard Evaluation System (SES) score of visual salt injury at the seedling stage for rice (IRRI, 1997). .....	30
Table 4: Rice varieties selected for the Vietnamese experiment .....	34
Table 5: Nitrogen and salt concentrations applied as treatments in Vietnam.....	36
Table 6: Number of days to attain the three-leaf stage sampling .....	40
Table 7: ANOVA models of the shoot length at the three leaves stage .....	40
Table 8: LSMEANS of the shoot length at the three leaves stage.....	40
Table 9: ANOVA models of the SES at 4 weeks of treatment.....	42
Table 10: SES values in strong salt conditions at 4 weeks of treatment. ....	42
Table 11: ANOVA models of the shoot dry weight at 4 weeks of treatment.....	42
Table 12: LSMEANS of the shoot dry weight at 4 weeks of treatment .....	43
Table 13: ANOVA models of the root dry weight at 4 weeks of treatment .....	44
Table 14: LSMEANS of the root dry weight at 4 weeks of treatment .....	44
Table 15: ANOVA models of the shoot length at 4 weeks of treatment.....	45
Table 16: LSMEANS of the shoot length at 4 weeks of treatment .....	46
Table 17: ANOVA complete model of the root length at 4 weeks of treatment .....	47
Table 18: LSMEANS of the root length at 4 weeks of treatment .....	47
Table 19: ANOVA models of the transpiration rate at 4 weeks of treatment .....	49
Table 20: LSMEANS of the transpiration rate at 4 weeks of treatment.....	49
Table 21: ANOVA models of the stomatal conductance at 4 weeks of treatment ...	50
Table 22: LSMEANS of the stomatal conductance at 4 weeks of treatment .....	51
Table 23: ANOVA models of the total protein content in shoot at 4 weeks of treatment .....	52
Table 24: LSMEANS of the total protein content in shoot at 4 weeks of treatment	52
Table 25: ANOVA model of the $\alpha$ -chlorophyll content at 4 weeks of treatment ...	55
Table 26: LSMEANS of the $\alpha$ -chlorophyll content at 4 weeks of treatment .....	55
Table 27: ANOVA model of the nitrogen content at 4 weeks of treatment .....	57
Table 28: LSMEANS of the nitrogen content at 4 weeks of treatment.....	57
Table 29: ANOVA models of the carbon/nitrogen ratio at 4 weeks of treatment ...	58
Table 30: LSMEANS of the carbon/nitrogen ratio at 4 weeks of treatment .....	59
Table 31: ANOVA models of the nitrogen use efficiency at 4 weeks of treatment .	60
Table 32: LSMEANS of the absorption NUE at 4 weeks of treatment.....	61
Table 33: LSMEANS of the physiological NUE at 4 weeks of treatment .....	62
Table 34: LSMEANS of the agronomical NUE at 4 weeks of treatment.....	64
Table 35: ANOVA model of the sodium content at 4 weeks of treatment.....	65
Table 36: LSMEANS of the sodium content at 4 weeks of treatment .....	65
Table 37: ANOVA models of the potassium content at 4 weeks of treatment.....	67
Table 38: LSMEANS of the potassium content at 4 weeks of treatment.....	67
Table 39: ANOVA models of the Na/K ratio at 4 weeks of treatment.....	68

Table 40: LSMEANS of the Na/K ratio at 4 weeks of treatment .....	68
Table 41: ANOVA model of the shoot dry weight at active tillering .....	70
Table 42: LSMEANS of the shoot dry weight at active tillering .....	71
Table 43: ANOVA model of the shoot length at active tillering.....	72
Table 44: LSMEANS of the shoot length at active tillering .....	72
Table 45: ANOVA model of the number of tillers at active tillering.....	73
Table 46: LSMEANS of the number of tillers at active tillering .....	74
Table 47: ANOVA models of the assimilation rate of CO <sub>2</sub> at active tillering.....	76
Table 48: LSMEANS of the assimilation rate at 4 weeks of treatment.....	76
Table 49: ANOVA models of the agNUE at active tillering.....	77
Table 50: LSMEANS of the agronomical NUE at active tillering.....	78
Table 51: Results of the anions content analysis (preliminary test) for the hydronics experiment .....	96
Table 52: ANOVA model of the shoot dry weight at heading stage.....	96
Table 53: LSMEANS of the shoot dry weight at heading stage.....	97
Table 54: ANOVA models of the agNUE at heading stage .....	98
Table 55: LSMEANS of the agronomical NUE at heading stage .....	98

## List of figures

Figure 1: Development stages of a 120-day rice plant in the tropics .....	7
Figure 2: Rice land ecosystems .....	9
Figure 3: Nitrogen transformations in a submerged soil under lowland rice .....	14
Figure 4: Salt tolerance evolution of the rice growth stages .....	20
Figure 5: Calendar of the experiment on the vegetative stage in hydroponics.....	29
Figure 6: Schedule of the Vietnamese experiment .....	35
Figure 7: Shoot length at the three-leaf stage .....	41
Figure 8: Shoot dry weight at 4 weeks of treatment .....	43
Figure 9: Root dry weight at 4 weeks of treatment .....	45
Figure 10: Shoot length at 4 weeks of treatment .....	46
Figure 11: Root length at 4 weeks of treatment.....	48
Figure 12: Transpiration rate at 4 weeks of treatment .....	50
Figure 13: Stomatal conductance at 4 weeks of treatment .....	52
Figure 14: Total protein content in shoot at 4 weeks of treatment .....	54
Figure 15: $\alpha$ -chlorophyll content at 4 weeks of treatment .....	56
Figure 16: Nitrogen content at 4 weeks of treatment .....	58
Figure 17: Carbon Nitrogen ratio at 4 weeks of treatment. ....	60
Figure 18: Absorption NUE at 4 weeks of treatment .....	62
Figure 19: Physiological NUE at 4 weeks of treatment .....	63
Figure 20: Agronomical NUE at 4 weeks of treatment. ....	65
Figure 21: Sodium content at 4 weeks of treatment .....	66
Figure 22: Potassium content at 4 weeks of treatment .....	68
Figure 23: Na/K ratio at 4 weeks of treatment. ....	69
Figure 24: Shoot weight at active tillering. ....	71
Figure 25: Shoot length at active tillering (5 weeks of treatment) .....	73
Figure 26: Number of tillers at active tillering (5 weeks of treatment) .....	75
Figure 27: Assimilation rate of CO <sub>2</sub> at active tillering.....	77
Figure 28: Agronomical NUE at active tillering .....	78
Figure 29: Shoot weight at heading stage.....	98
Figure 30: Agronomical NUE at heading stage.....	99



# Introduction

Rice is a major food supply in the world, especially in Asia where it represents from 20% to 65% of the daily caloric intake (CGIAR, 2013), and is one of the three most cultivated crops currently with wheat and maize. The challenges for rice production are substantial in order to keep on supplying an increasing global demand. One of them is to ensure and contribute to food security in developing countries. A second challenge is to face the climate change and its consequences on the rice crop science. The impacts will be a global warming with the rising of the sea levels, more droughts, floods, salinity stress and a higher frequency of extreme climatic events. Salinity is a significant preoccupation for rice production due to the rising sea levels and salt intrusion in the lands, especially in Vietnam where most of the irrigated fields are cultivated in the deltas. This will have a repercussion on the rice arable area, which will decrease or be less productive in the future. The most important challenge is therefore to improve the rice yield in a more sustainable way and to be more resilient to climate change.

Nitrogen is a fundamental element for the plant metabolism and a determinant one for the yield determination. The related literature is abundant but still several mechanisms remain to be studied in order to improve the rice management and productivity. In this context, an adapted agronomical indicator, the Nitrogen Use Efficiency (NUE) has been used. It characterizes the efficiency of the nitrogen uptake and its utilization by the plant for its growth. This tool can allow the quantification of an optimal nitrogen application under various conditions such as salinity. The mechanisms of this interaction between the salt stress and nitrogen still have to be understood.

In that regard, this master thesis aspires to contribute to a better characterization of the interaction between rice NUE and saline conditions. It is part of the PhD research project of Nhung Phan (VNUA/UCL) and is continuing the master thesis of Adrien Heymans (UCL, 2017).

As rice under salt stress seems to respond differently depending on the nitrogen fertilization, several experiments were made to identify and validate the trends of this interaction. The first part of this work consisted of two experiments conducted in hydroponics inside a phytotron at UCL. They had for objective to isolate trends in the interaction during vegetative phase between three fixed parameters (salt-tolerant or sensitive varieties, different salt levels and decreasing nitrogen concentrations). The second part consisted of an experiment in pots in Vietnam and intended to validate those trends to closer field conditions at the Vietnam National University of Agriculture.



# Chapter I: State of the art

## 1 Rice

### 1.1 Generalities

Rice is one of the third most cultivated cereals in the world with nearly 741 Mtons for an area of 160 Mha in 2016. According to the predictions and current statistics, the productivity increases due to the improvements of the techniques and materials (Kabir et al., 2015), even if it will be on a slower rate than in the past 60 years (Adjao et al., 2015). On the other side, the cultivated area will be stabilized to the current surface or could decrease a bit like it has been the case since 2013 (5M ha less). Indeed, rice is produced worldwide with 90 percent from Asia, where the urbanization is still growing and therefore the land use is changing, declining arable land (Chauhan et al., 2017). This phenomenon of rice fields losses is also a consequence of the increasing seawater intrusion on the land (Takeuchi et al., 2018). Vietnam is the fifth rice producer with 43 million tons (5.8% of the world production in 2016) and is also the fifth most important exporting country (7.5% of the world exportation in 2017). This is the result of the agriculture intensification set in by the government since the green revolution and mostly since the nineties (Workman, 2018). The Vietnam average rice yield was of 5.5 tons ha<sup>-1</sup> when the world average yield is of 4.6 tons ha<sup>-1</sup> in 2016 (a rice yield can attain 10 tons ha<sup>-1</sup> and more). An interesting trend is that the contribution of rice toward total calorie intake is declining in Asia with the diversification of the diet with high-value products such as meat (30.2% in 1967 to 26.8% in 2007 for China) (Timmer, 2010). Even if the trend is also applicable - but with a slower decrease - in Vietnam and the Southeast Asia countries, rice is still the major intake over there with 60% of the daily calorie consumption (FAOSTAT, 2018).

Rice belongs to the *Poaceae* family and is divided in two main cultivated species, one originated from Asia and cultivated worldwide, *Oryza sativa* L., and the other, *Oryza glaberrima* Steud., from Africa and cultivated only on that continent (more rustic). *O. sativa* has two major subspecies, *japonica* from China and *indica* from India. *Indica* rices are grown throughout the tropics and subtropics when *japonicas* are limited to temperate regions. Traditional *indicas* have a poor response to a high nutrient input conditions, they are tall with a high tillering capacity, droopy leaves and long grains with a high amylose content (dry and flaky when cooked) but the stem is weak, causing lodging problems. *Japonicas* have short and stiff stems, producing round grains with a low amylose content (sticky when cooked). They are highly responsive to nutrient inputs (Prasad et al., 2017). The genus *Oryza* is composed of 11 groups labeled AA to LL. Most of species of interest like *O. sativa* and *O. glaberrima* are diploid (AA genome with 12 chromosomes) but other ones can be allotetraploid (GRiSP, 2013).

*O. sativa* has a cycle achieved in three to six months depending on the environmental conditions and the cultivar. The cycle is composed of two main growth phases (the vegetative and the reproductive phase), to which can be added the ripening phase, as explained in the subsection 1.3. For a 120-day rice variety, the classic scheme of those phases is distributed in 60 days for the vegetative stage, 30 days for the reproductive one and finally 30 days for the ripening. This cereal is a C<sub>3</sub> monocotyledonous plant adapted to hot environment. The disadvantage of the photorespiration on C<sub>4</sub> plants in hot weather is bypassed by the need of a high-water availability, causing an important evapotranspiration and leading to a compensation of the less efficient photosynthesis. This optimal photosynthesis is reached at temperatures of 25 °C to 30 °C, even if the extreme rice temperatures are from 10 °C to 50 °C (De Datta, 1981).

Rice is produced in all of the six continents where field crop production is practiced, and on a large range of environments and soils, from loamy sands to heavy clay loam or clays. It can be grown on acid soils from a pH of 4 to sodic ones of pH of 9 with an optimum around 6-7. Regarding the organic matter in the soil, the same observation can be made with rice being cultivated with less than 1% of organic matter to 95%. The most important condition for the classic production is to have a waterlogged soil (Chauhan et al, 2017; De Datta, 1981).

One important characteristic of traditional rice is the photoperiod dependency, it is a short-day plant. The vegetative phase is divided in two stages depending on the photoperiodism. The first one, called the basic vegetative stage, is insensitive to the photoperiod and the second one, called the photoperiod-sensitive phase, is the stage influenced by the photoperiod and corresponding to 35 days before the flowering (Yoshida, 1981). This mechanism is set to able the plant to have a response to the environmental conditions. Once the photoperiod-sensitive phase is reached, the initiation of the panicle primordia is made when the day length is reaching or falling down the optimum photoperiod of 9-10 hours. A longer photoperiod than the optimum will cause a delay in the initiation. Rice will be able to initiate the flowering until the critical photoperiod, which is of 12 to 14 hours. Nowadays, a lot of improved varieties are independent to photoperiodism in order to diminish the duration of cultivation and therefore making the cultivation of several crop per year feasible.

The water needs for rice cultivation are high in comparison to other cereals. A seasonal water input is demanding between 660 and 5280 mm varying with the environmental conditions. The repartition is of 160-1580 mm for land preparation (as the puddling explained in point 1.4.1), 400-700 mm for evapotranspiration and 1500-3000 mm if it is sandy/loamy soil or 100-500 mm if it is a heavy soil for the losses due to percolation and seepage (Tuong et al., 2003). The average water input for irrigated rice in Asia is of 1300-1500 mm and it takes 1432 L of water for 1 kg of rice (IRRI rice

knowledge bank, 2018). So, the main part of the water needs is to compensate for the losses of seepage and percolation (25 to 85% of the water input).

## **1.2 Morphology of the Rice Plant**

*Oryza sativa* is a semiaquatic annual grass, although it can survive as a perennial in the tropics by producing new tillers (culms) after the harvest (ratooning). The rice plant is composed of the main stem joined to a number of culms called tillers. Each stem is round, with flat leaves and usually a terminal panicle at maturity. The stem is composed of a series of nodes and internodes. A node bears a leaf and a bud, which can grow into a tiller. The internode length varies depending on the variety and environmental conditions, even if it generally increases from the lower to the upper part of the stem. Generally, the number of nodes varies from 13 to 16, mainly regrown in shorts, thickened section (internodes of less than 5 mm), with only elongating internodes on the 3 to 8 upper nodes (elongation is considered when an internode is longer than 5 mm). The length of the lower internodes can also be adapted to the growing conditions, like if an early water level is increasing importantly (a low internode of a deepwater rice variety can reach a maximum 25 cm/day elongation). The plant height goes from 0.4 m to more than 5 m in some floating rices (Yoshida, 1981; GRiSP, 2013). The lowest internodes elongation is often the main reason of the lodging phenomenon, when the panicle weight is high with the grain filling. The elongation, coupled with the low strength or stiffness of the stem, can be caused by an excess of nitrogen application for the high yield varieties.

The leaf blade is attached at the node by the leaf sheath encircling the stem. A hairy auricle can be seen at that junction just under the ligule. The last leaf before the panicle is called the flag leaf and is determinant for the grain filling, as for other cereals like wheat. The outer diameter of internodes varies from 2 to 6 mm. The main center part or inner diameter, in a crossed section, of the internode culm is composed of a large hollow space called the medullary cavity. In addition, many large vascular bundles and air spaces are found in the internode tissue. This aerenchym is very important to gas exchange of the plant (Yoshida, 1981).

The panicle is an indeterminate inflorescence developed around an axis going from the base to the apex of it. The axis is composed of 8 to 10 nodes at 2-4 cm intervals, where the primary branches are developing. Secondary branches are developing in the same way on the primary branches. Finally, growing from the nodes of the primary and secondary branches are the pedicels, with the spikelets positioned above them. Only one complete floret (flower) is produced per spikelet and is protected by rudimentary glumes (De Datta, 1981; GRiSP, 2013).

As for the root system two main types may be distinguished. The first type is the seminal roots growing at the germination from the radicle on maximum 15 cm. They are

important at the germination but are quickly replaced by the second type of roots, the nodal roots (or adventitious). They are produced from the lower nodes (5-25 roots node<sup>-1</sup>). The primary nodal roots can grow to 1 m or more in soft upland soil with a diameter ranging from 0.5 to 1 mm, but in flooded soils, the length seldom exceeds 40 cm due to the difficulties of the oxygen diffusion to supply the growing root tips. The aerenchym presence in the roots, caused by a programmed cell death inside the cortex and connected to the culm's ones, is preponderant to the gas diffusion and the root growth. Rice can develop roots until the sixth-order branched roots. The hair root formation is favored by aerobic environment in upland soils (Yoshida, 1981).

### **1.3 Physiological Phases of Rice**

As said above, growth and development cycle durations of rice are variable from 3 to 6 months, depending on the variety and environmental conditions. In the case of this subsection, a 120-day duration will be taken as a reference for the development of the rice plant like it is schematized in the Figure 1: Development stages of a 120-day rice plant in the tropics (GRiSP, 2013). The three physiological phases are the vegetative, the reproductive and the ripening phase. The vegetative one, which lasts 60 days, is subdivided into the germination, the early seedling growth and the tillering. The reproductive phase, of 30 days, is subdivided into the pre-heading or the panicle initiation, its development and the flowering. Finally, the ripening phase, lasting 30 days also, is composed of the grain filling and maturation of the panicle (GRiSP, 2013; De Datta, 1981; Yoshida, 1981).

The vegetative stage is meant to accumulate nutrients, develop the most efficient architecture for the photosynthesis, in order to reallocate the reserves and favorize a good reproduction. The main part of the nutrients will be collected before the heading. This phase is characterized by a gradual increase in plant height, a regular interval between leaf emergence and the active tillering. The first tiller will appear after the 5-6<sup>th</sup> leaf on the main stem, then the tillers emergence will follow a similar pattern. The number of tillers will be maximum at the end of the vegetative phase, which is corresponding to the panicle initiation (Yoshida, 1981).

The reproductive phase is characterized by culm elongation, the regression of the ineffective tillers (with no future panicle) to reach the final number, the emergence of the flag leaf, but mostly of the panicle primordia initiation, the booting, heading and the flowering. For the photoperiodic varieties, the initiation is combined with the optimum day length (subsection 1.1). The spikelet anthesis (flowering) begins just after the heading, during the morning and the fecundation is usually completed in six hours, but can be made within 5 days (De Datta, 1981).

The ripening phase is a continuous process and the grain undergoes distinct stages before maturity. The grain goes through the milk stage, where the starch portion is turning from a watery to a milky consistency. Then, the dough stage is reached with the milky starch becoming a hard dough. The last stage is the maturation one, the grain becomes mature and yellow, as the rest of the plant. The senescence of the upper leaves can be observed at this time (Yoshida, 1981).

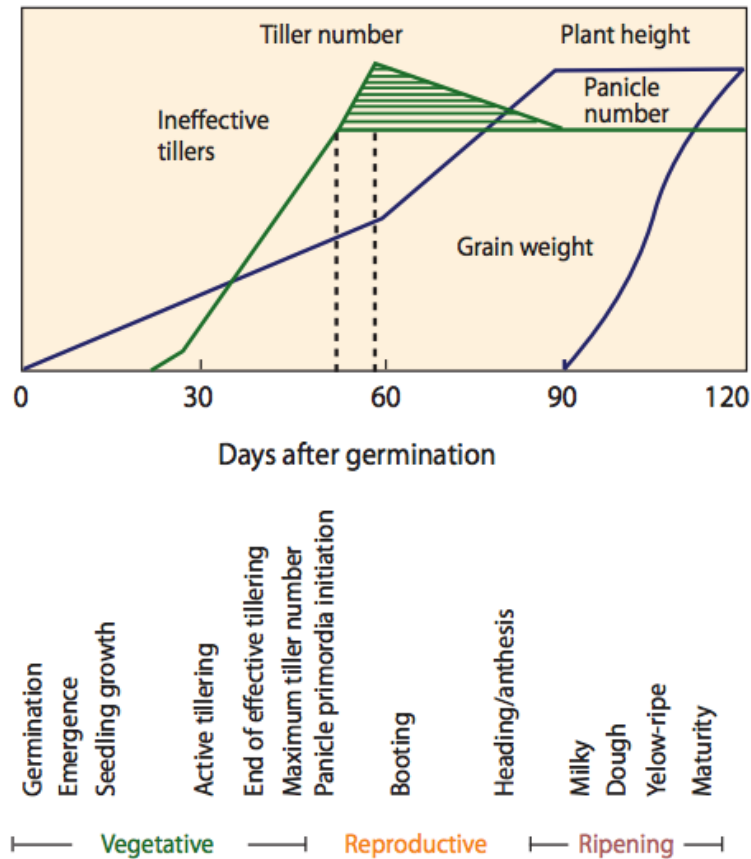


Figure 1: Development stages of a 120-day rice plant in the tropics (GRiSP, 2013)

## 1.4 Rice Ecosystems

### 1.4.1. Generalities

Rice is grown under a large range of ecosystems. Four rice field ecosystems (RFES) have been categorized by the IRRI: upland, irrigated, rainfed lowland, and flood-prone rice ecosystems (Figure 2). The characteristics of the ecosystems, such as the plant nutrients need, the management strategies, the control of the weeds and pests, are different from each other (De Datta, 1981, Prasad et al., 2017; GRiSP, 2013).

The rainfed upland RFES is usually categorized by various types of fields, from leveled to steeply sloping ones, but with high runoff. This system is present in all kinds of climates, from humid to sub-humid, with soil relatively fertile to highly infertile. The fields are rarely flooded, and the soil is in aerobic conditions. The rice is directly sown in dry plowed soil or transplanted in wet but non-puddled soil. The productivity is usually really low ( $1 \text{ t ha}^{-1}$ ) but can although reach consequent yield ( $5 \text{ t ha}^{-1}$ ). This rice ecosystem covers 10% to 12% of the world's rice land.

The irrigated RFES, is the most efficient ecosystem for growing rice. The fields are leveled and bounded to control the water level. In this system, the water supply is assured for one or more crops a year (in continuous conditions, two or three crops/year). The rice is transplanted or directly sown in puddled soil (point 1.4.1). The cereal is developing in anaerobic and controlled shallow flooded conditions (5-10 cm depth) during all the crop growth duration. Irrigated lands cover more than 50% of the world's rice land and are responsible for 75% of world production. It's receiving 40% of the world's irrigation water.

The rainfed lowland can be submitted to flooding or drought problems. The fields are bounded, nearly leveled or have a slight slope and are grown in river deltas or in coastal areas. The flooding is usually non-continuous, depending on the precipitations, with variable water depth and duration. Abiotic stresses can be caused by this water variation, and salinity stress can be observed in this ecosystem. The submergence cannot exceed 50 cm for more than 10 consecutive days. Rice is usually transplanted or directly sown in puddled soil. It also can be directly sown in dry plowed soil. In this ecosystem, an alternation of aerobic and anaerobic soil conditions is present. The rainfed lowland covers 20% of the world's rice land.

The flood-prone RFES is subjected to uncontrolled flooding due to its proximity of the valley's bottom and the river. The field is leveled or with a slight slope and can be depressed. The water conditions can be extreme with submergence lasting up to 5 months during the crop growth, with a water depth from 0.5 to 4 m. Rice is, as in the rainfed lowland, transplanted or directly sown in puddle soil, but also directly sown in dry plowed soil. Depending on the water presence, the plant can grow in aerobic or most of the time in anaerobic conditions. A particularity of this ecosystem is the possibility of the soil salinity in tidal areas. This system covers 8% of the world's rice area.

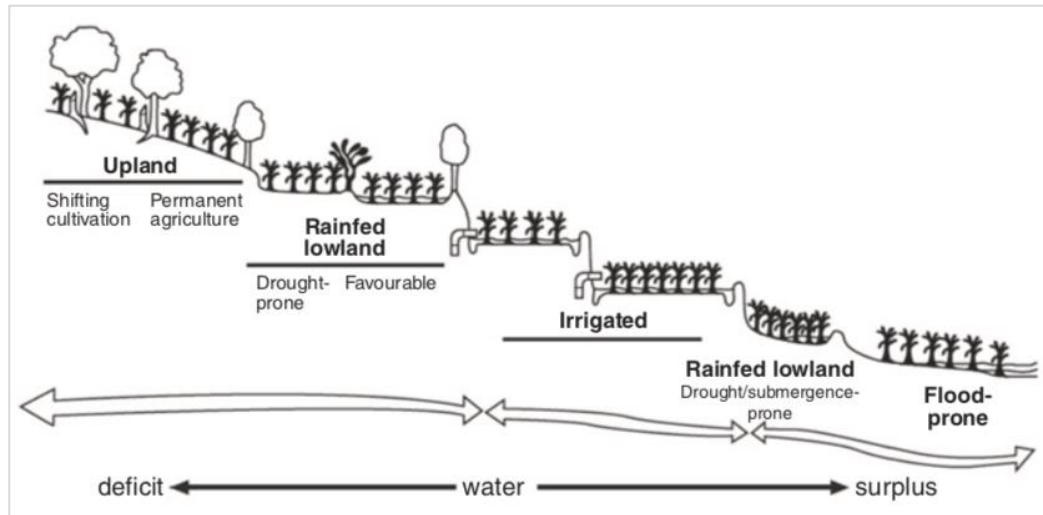


Figure 2: Rice land ecosystems (after Greenland 1997 as adapted from IRRI 1993)

### 1.4.2. Puddled Soils

Puddled soils are used for the majority of the rice production in order to keep waterlogged fields condition. The word *puddle*, defined by Rice in 1943, means a clay that can be worked to a water-impermeable stage. Puddling consists to destroy the soil structure (91-100% of the macropore volume) and restrict the porosity to a top layer. This process can be done only on soil with more than 20% of clay. The interest of puddled soil has been shown with the reduction of the water needs by two and the limitation of the percolation and seepage losses by three in comparison to non-puddled soil. The gas exchanges are low in this anaerobic soil and the reductive reactions are enhanced (De Datta, 1981).

Before starting the puddling, the fields are delimited with bounded borders. A network of canals and drains are created to control the water level. The puddling is begun with a primary tillage by plowing, following by a secondary one by repeated harrowing, to make the impermeable layer. Some animals or mechanical devices can be used to mix the soil in order to destroy its structure.

One of the advantages of paddy fields, beside the water retention, is the easy weed control caused by this method. Another advantage is the favorable mud soil for the transplantation. The incorporation of the basal dose of fertilizer is also facilitated by the mix. A disadvantage of this land preparation practice is the requirement of an important water amount (160-200 mm in a wetland field). The total water use in a non-puddled field is 50% of the quantity for a wetland field despite the longer crop duration of the first one (De Datta, 1981). The rainfed ecosystems are more subject to the water constraint to make paddy fields, because the farmers need to wait 1-3 months in order to

have sufficient water for puddling. Paddy fields also complicate the implantation of the next crop (other than rice), which are not adapted to these anaerobic soil conditions.

In comparison with direct sowing, transplantation is the most associated system to paddy fields. It allows to make germination in nurseries, and therefore concentrate the seedlings to gain space, water and seeds. Germination can be made simultaneously to the puddling. Another advantage of the transplantation is the roots enhancing and tiller development. A disadvantage to the direct sowing is the repetition of labor at the beginning of the crop season, and the one-week delay for the rice maturity (Yoshida, 1981).

## **1.5 The Future of Rice**

### **1.5.1. Impact of Climate Change**

The major impact of the climate change is characterized by the global warming and the rising of the greenhouse gases (GHG). The effects of the climatic variations will be the threatening of the productive capacity of rice ecosystems by an increasing water scarcity in irrigated systems, by droughts, salinity, uncontrolled flooding and other extreme events that will be more frequent (Chauhan et al., 2017). The rising of the sea level is a major preoccupation for coastal regions and particularly for the salinity intrusion in crops. An estimation has been made for the Vietnam East Sea, and the results show, in the best scenario, that the sea level will rise with an average of 52 cm by the end of the century (Tran et al., 2017).

In a simulation study, an increase of 2 °C has shown a loss of 3-10% in the yield of a crop like rice and causes a decrease of 4-9% of the maximum LAI of the plant (Prabhjot-Kaur et al., 2006). Moreover, it has been highlighted that the increasing temperatures or hotter night temperatures can reduce grain yield by the diminution of the spikelet fertility (Wassmann et al., 2007) and translocation to the grain. Indirectly, a rising temperature has an effect on the weed population, the growth rate should be faster and their diversity higher, with a consequence of a harder weed management and the adaptation of it (Chauhan et al., 2014).

The water scarcity is a major threat. It has been estimated that, by 2025, 15 to 20 million ha of irrigated rice will suffer from water deficit (at different levels). It is also expected to cause a crop diversification among the water-short areas, where aerobic soil conditions will be present (GRiSP, 2013). Other consequences will occur in non-flooded fields, with more frequent aerobic soil conditions, where the growth and diversity of weed will be promoted. It's also the case with more frequent presence of

soil-born pests, diseases and micronutrient disorders. An adaptation of the pesticides will have to be made to deal with that problematic (Chauhan et al., 2014).

The quality of the grain will be altered as an effect of the climate change. It has been shown in several researches that the amylose content in rice grain (determinant in the cooking quality) is increasing under higher concentrations of CO<sub>2</sub> (Conroy et al., 1994). Higher CO<sub>2</sub> concentrations are leading to a reduced uptake by the plant of nitrogen, zinc and iron, with a consequence of reducing the rice nutritional value (Taub et al., 2008).

An important point to highlight, even if it is not a direct impact but is a part of the process, is the evident participation of the rice cultivation to the emissions of greenhouse gases. The main gases produced are methane (CH<sub>4</sub>) in anaerobic soil conditions and nitrous oxide (NO<sub>2</sub>) due to the nitrification-denitrification processes when alternating moist and dry soil conditions (Weller et al., 2014). The methane emissions from rice are estimated to reach 5-19% of the total world emission of this gas (Denman et al., 2007).

### **1.5.2. Rice Response Options for the Future**

With ecosystems threatened, it is imperative to find adapted and mitigated strategies to keep a sufficient rice production. The solutions have to come from the genetic research, but also from agronomic management practices.

In the short term, the yields of the current rice can still be improved with the mechanization and the agronomic management practices, such as an efficient nitrogen fertilization. This can be made in major rice producing countries, like in Southeast Asia, where there is still a gap between the current yields and the potential ones. In other more developed countries, such as China and Japan, the gap is thin and therefore a yield increase is more going through the genetic amelioration and improved varieties. Those improvements have to be technically viable, socially acceptable, environmentally integrated and economically adapted to farmers in order to be sustainable. Nevertheless, studies have shown that the potential scope of water saving from the total water input (irrigation, rainfall) management is far more efficient than the possible gain through the plant transpiration efficiency (Molden et al., 2010). Therefore, genetic improvement is not the only solution to the future and the climate change.

Traditional breeding programs have been directed to select varieties tolerant to salinity, drought, submergence or an aerobic soil, the wet-dry soil alternation, or compatible with diverse interesting techniques like direct seeding. In addition, physiological, biochemical, molecular aspects like quantitative trait loci (QTL), mi RNA technology and transgenic approaches are new tools to improve rice productivity. The identification of QTLs is an efficient way to select new varieties based on their

genome. A lot of studies are using this technique, such as the research of heat tolerance during flowering (Ye et al., 2015) or the capacity of drought tolerance during the critical stages (flowering and grain filling) for a final yield advantage of 0.7-1.2 t ha<sup>-1</sup> over normal varieties (Kumar et al., 2014). Many studies have been made on the salinity tolerance, but this point will be developed in the section 4 on the salinity.

Prospective research from the international C4 rice consortium (IRRI and partners) are looking for the feasibility to transform the rice in a C4 plant. This genetic transformation could be outstanding and change all the agronomic approach. The improvement of the yields could reach an increase of 30% to 50% in irrigated and rainfed lowland ecosystems (Kajala et al., 2011). However, this hypothetical transformation has been in evaluated for many decades without significant progress.

## **2 Nitrogen**

### **2.1 Nitrogen Cycle**

Nitrogen is the fourth most abundant element in cellular biomass. It's a major constituent of amino acids, proteins (enzymes), nucleic acids, and chlorophyll in plants. It's usually the limiting nutrient for a crop production. Although nitrogen is very abundant in the atmosphere as dinitrogen (N<sub>2</sub>), it's not directly available for the plants under this form and has to be converted. All the nitrogen is part of a dynamic cycle on every scale, from the atmosphere to the intracellular level. The cycle is composed of the nitrogen fixation, nitrification, denitrification and mineralization. To those main processes, additional ones occur in agriculture: volatilization, leaching and lixiviation (De Datta et al., 1989; Bijay et al., 2017).

The nitrogen fixation from N<sub>2</sub> gas in the atmosphere to give ammonia (NH<sub>3</sub>) is a reduction reaction made by organic fixation (bacteria or archaea with nitrogenase enzymes), or with abiotic process, such as the industrial Haber-Bosch fixation, or by lightning geochemical fixation.

The nitrification is the process to convert ammonia to nitrite (NO<sub>2</sub><sup>-</sup>) in first place and to nitrate (NO<sub>3</sub><sup>-</sup>) in second place. This oxidation process is made in aerobic conditions exclusively by prokaryotes. Those processes are distinct by the two different reactions with specific enzymes.

The resulting opposite reaction, converting nitrate to dinitrogen gas in anaerobic conditions by bacteria, is called denitrification. An intermediate form of this process, nitrous oxide (N<sub>2</sub>O), is an important greenhouse gas, specifically for the rice production.

Mineralization (ammonification) is made by the decomposition of the organic nitrogen in ammonia (inorganic). Immobilization is the opposite reaction.

## **2.2 Nitrogen Transformation in Flooded Rice Soils**

Rice is grown mainly in anaerobic conditions with waterlogged soil, but the soil moisture regimes during the crop season can be variable and dynamic. Aerobic or anaerobic conditions have an influence on the nitrogen forms and the pace of the different processes. This has an impact on the nitrogen fertilization management in rice production, where only 30-40% of the applied N can be used by the crop (Raun and Johnson, 1999).

In a flooded field, the denitrification process is enhanced as anaerobic conditions are present (Figure 3). First of all, under aerobic conditions, NH<sub>4</sub><sup>+</sup> is rapidly oxidized to nitrate, which will be diffused in the anaerobic layer, or is volatilized in the atmosphere (losses about 20-56% of N applied) (De Datta et al., 1989). In the anaerobic layer, all the nitrates are quickly lost by denitrification (12-33% of nitrogen losses) and leaching (Aulakh et al., 2001). Therefore, nearly all the ammonium transformed into nitrate inside the aerobic layer is lost before its uptake by the plant (Buresh et al., 2008). The optimum fertilization is to use some nitrogen on ammoniacal forms like urea (CH<sub>4</sub>N<sub>2</sub>O). NH<sub>4</sub><sup>+</sup> is the most available form for the plant uptake in waterlogged conditions. Urea has to be applied in the anaerobic layer of the soil to optimize the plant uptake and to delay as much as possible the nitrification-denitrification process. Nevertheless, it can be lost by volatilization, clay minerals fixation (NH<sub>4</sub><sup>+</sup> fixation capacity of a soil has to be taken into account to maximize NUE). The nitrogen from the immobilization process is partially lost as the mineralization of the soil organic matter is returning nitrogen to soluble NH<sub>4</sub><sup>+</sup> continuously. To optimize the plant uptake, the applications of fertilizers should be coupled to the mineralization rate (Bijay-Singh et al., 2017). The pH also has a role in the processes and needs to be observed. A high pH is increasing the volatilization and a low one is decreasing the mineralization (De Datta et al., 1989).

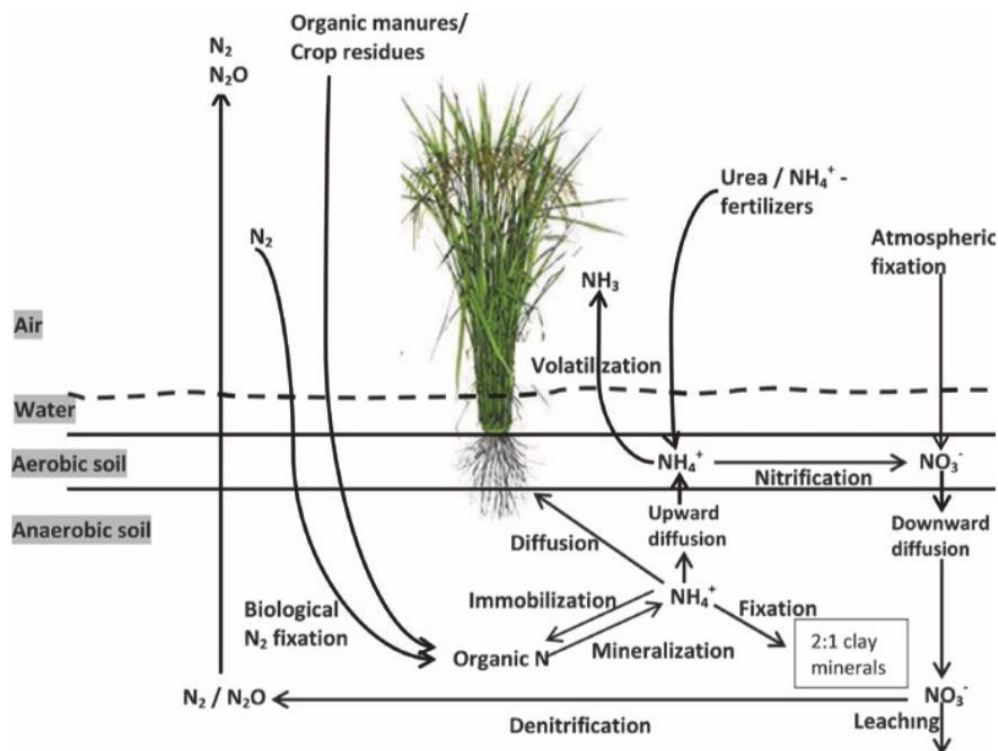


Figure 3: Nitrogen transformations in a submerged soil under lowland rice (Bijay-Singh et al., 2017)

## 2.3 Rice Nitrogen Uptake

As it is explained in the previous point, nitrogen can be found in various forms in the rice soil solution. As most of the plants, rice can absorb nitrates ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ), and the preferential N form uptake is chosen by its concentration pool. In the case of rice, the privileged form is ammonium as it is the most stable form and as the fertilization management is focused on preventing the nitrification. However, this prevention is the main cause of the poor nitrate absorption (Buresh et al., 2008). Various studies have shown that the absorption by rice is more efficient during the vegetative stage for the ammonium, while the nitrate is more efficiently absorbed during the reproductive phase (Takenaga, 1995). The uptake of nitrates can be underestimated, substantial quantities of  $\text{NO}_3^-$  could be absorbed and be comparable to the ammonium absorption (Kirk and Kronzucker, 2005). For an optimized N uptake by the plant, nitrogen should be applied under the ammonium form at the beginning of the crop, then under the nitrate form at the initiation of the panicle. Finally, a topdressing of any form is equally efficient (De Datta, 1981; Wilson et al., 1994). The ratio  $\text{NH}_4^+/\text{NO}_3^-$  ion uptake can modify the rhizosphere pH up to 2 units compared to the rest of the soil. An important ammonium concentration lowers the pH, while a high nitrate level rises the pH of the soil solution. Urea can be substituted by ammonium sulfate fertilizer  $[(\text{NH}_4)_2\text{SO}_4]$ , it is a better source of nitrogen than urea on alkaline soils because ammonium sulfate is more efficient in reducing the soil pH (Rakshit et al., 2015).

Two major nitrogen uptake and assimilation pathways ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in rice are well known. Rice roots absorb the nutrients with a wide range of transporters. The absorption of ammonium appears to be faster than the one of nitrate (Gaudin and Dupuy, 1999). In addition, after the uptake, the nitrates have to be reduced to ammonium by nitrate and nitrite reductase for the assimilation (Shi & al., 2010). The quantity of energy necessary for the assimilation is higher for  $\text{NO}_3^-$  (equivalence to 20 ATP mol<sup>-1</sup>) than for  $\text{NH}_4^+$  (equivalent to 5 ATP mol<sup>-1</sup>) due to the reduction (Salsac et al., 1987). Therefore, potential energy savings could be made with a strict ammonium fertilization. An ammonium assimilation occurs in roots and shoots with the incorporation by glutamine synthetase and glutamate synthase (Fageria, 2008).

An important property of nitrogen is its mobility inside the plant. Therefore, it can be reallocated to sink organs such as the panicle. It can also be transfer to the new leaves in case of N deficiency. The deficiency symptoms are first appearing on the older leaves.

During active tillering, the nitrogen availability is determinant and is highly responsible for an optimum growth and yield. With a good availability of nitrogen, almost half of the final N content is absorbed, and half of the dry matter is produced before the panicle initiation. About 60-70% of the shoot nitrogen is located in the panicle at maturity after the remobilization of N (Yoshida, 1981; Bijay-Singh et al., 2017).

## **2.4 Functions of the Nitrogen**

As it is well known, nitrogen is one of the most significant elements for the growth and yield of plants. It has a center role for many physiological and biochemical processes and can be found in many important organic compounds from proteins to nucleic acids. Nitrogen is a constituent of the chlorophyll and therefore has a major influence over the photosynthesis. Proteins are quaternary compounds divided in two classes: the enzymes regulating metabolic reactions and the constitutive proteins (cell walls, membrane ...). The nitrogen content and repartition in a plant is in function of the plant physiological status.

The interaction between nitrogen and the plant growth is well explained by the importance of nitrogen in the constitution of the chlorophyll and Rubisco, which are running photosynthesis. The N uptake positively influences the photosynthesis activity. Therefore, the plant growth is enhanced (with an increasing LAI) by the photosynthesis activity, and the nutrient uptake is promoted. The mature rice leaf chloroplasts contain up to 60% of the leaf nitrogen, mainly in the Rubisco and the chlorophyll (Morita 1980). More specifically, during the leaf growth, Rubisco varies from 22% to 32% of the total leaf N content and is representing 50% of the soluble proteins (Makino et al., 1984;

Evans, 1989; Lawlor, 2002). However, the nitrogen ratio for other photosynthetic components in proportion to the total N leaf stays constant (Mae, 1997). A nitrogen deficiency decreases the soluble protein content and the protein synthesis per leaf. The structural proteins aren't subject to that reduction, and thus the ratio of structural/nonstructural protein increases. For the same reason, this deficiency decreases the leaf chlorophyll content, thereby changes the proportion of light-absorbing pigments and reduces the photosynthesis activity.

## 2.5 Nitrogen Use Efficiency

The Nitrogen Use Efficiency (NUE) has been defined as an indicator of the plant efficiency to use the nitrogen. Several definitions are found in the literature. It can be classified in output/input ratios as absorption efficiency (aNUE), physiological efficiency (pNUE), and agronomical efficiency (agNUE) (Fageria, 2008).

$$aNUE [\%] = \frac{N \text{ absorbed by the plant } [g N]}{N \text{ applied } [g N]} \times 100$$

$$pNUE = \frac{\text{Total dry weight } [g]}{N \text{ absorbed by the plant } [g N]}$$

$$agNUE = \frac{\text{Total dry weight } [g]}{N \text{ applied } [g N]}$$

NUE is a valuable tool to quantify the N productivity of the crop. In the case of rice, NUE has a high variation depending upon the fertilizer source, the method of incorporation, the rate and application time. The recovery efficiency in rice varies from 20 to 80% with an average of about 30 to 40% (Cassman et al., 1993). However, even if most of the non-absorbed N is lost by denitrification, volatilization or leaching, a significant proportion is immobilized in the soil organic matter with N incorporation by the microbial biomass. It has been shown that 16-25% of the total applied nitrogen is recovered in the soil organic fraction after the panicle differentiation of the rice (Bollich et al., 1994). A low N availability in the soil is increasing the NUE of the crop. Usually, the NUE is higher for the other cereal crops than for the rice, due to the flooded conditions.

A great challenge for the future is to improve the NUE, in order to either improve the biomass (and hopefully the grain yield) under constant N application or to maintain the biomass and grain yield under reduced N application., by adopting appropriate crop

management practices in function of the plant status, the soil conditions, the climatic conditions and by using genetics to select new varieties (Fageria, 2008).

## 2.6 Fertilizer N Management in Rice

The fertilizer N management in rice is crucial for an efficient N uptake. The management has to be set on the critical growth stages and the particularities of the rice crop ecosystem. Thus, four parameters of fertilizer N management can be highlighted as the source, rate, time and place. They are interconnected between each other and with the agricultural practices. More than one combination of the parameters can be efficient for a given situation in a crop. The N-fertilization is usually split at least in two or three doses for all kinds of rice system. The application of an ammonium-formed N fertilizer deep in the anaerobic soil conditions prevents the losses due to volatilization. The applications are coupled appropriately with the other main nutrients such phosphorus and potassium (Bijay-Singh et al., 2017).

The first application, the basal dose, made of an ammonium forming source, is allocated usually with 50% of the total N applied. The application technique for a direct rice seeding system varies from the transplantation rice system. The application in the first system is made during the early vegetative growth with the irrigation following it. The fertilization of the second system is applied before transplantation and can be administered in different ways; to the soil surface and incorporated by the flooding (most used technique), to the soil surface and incorporated mechanically before the flooding and finally injected into the soil.

The next doses, applied as top dressings generally at the active tillering and panicle initiation (25% of total N applied each), are more efficiently absorbed by the plants. The recovery efficiency is higher than for the basal dose. However, the top-dressing doses can be variable as they are more subject to the growth stage and the time of application. Indeed, as the proportion of total nitrogen applied is lower, a root system well developed and a high plant nitrogen requirement at this stage, the indicator is very elevated with a range between 45 and 82% (Westcott et al., 1986; Wilson et al., 1994).

A good way to synchronize the nitrogen crop demand and the availability from the fertilizer applied in order to increase the NUE and reduce N losses is to use *controlled-N release fertilizers*. It is consisting of highly soluble urea granules coated with a water-insoluble layer like sulfur or polyolefin. The rate, pattern and duration of the nitrogen release is controlled by the dissolution of this coat. Those characteristics allow to reduce the number of applications with the optimal and slower N release, constituting an interesting economy of labor (Shaviv, 2001; Bijay-Singh et al., 2017). NUE has been increased by 3 to 34% and the yield by 5-16% with the utilization of controlled-N release urea (Li et al., 2018).

Another management practice about the N-fertilization is the utilization of *urease* and *nitrification inhibitors*. Urease inhibitors, such hydroquinone, prevent the ammonium volatilization (about 20-56% losses of N applied) from the urea hydrolysis (Bijay-Singh, 2017). Nitrification inhibitors delay the transformation of the ammonium in nitrite by inhibiting or slowing the activity of *Nitrosomas* bacteria. Studies have shown that the application of a nitrification inhibitor product (dicyandiamide - DCD) has increased the rice yield of 16.5% (Linguist et al., 2013). The inhibitors are added to the fertilizers during the applications.

## **2.7 Misuse of Nitrogen**

Even if the research to improve the rice cultivation and the NUE is crucial for the future, the final decision about the management of the N-fertilization is taken by the farmer. Usually in Asia, farmers work on small cultivation area and investments are limited by their economic situation. Therefore, the traditional farming practices are followed without integrating new recommendations due to an inefficient transmission of the information in the countryside. The result is often the overuse of the nitrogen that is leading to important N losses and in the worst scenario to the water eutrophication. An important source of N misuse is the inappropriate combination of the four N fertilization parameters, resulting in a desynchronization of the availability/demand and a low NUE. On the other hand, the farmers could be very flexible about the time of the application and the amount of fertilizer applied, as the exploitation area is limited. Thus, by applying a site-specific N management, the quantity of fertilizer is adapted to the soil conditions, the crop status and is taking into account the field to field variability in order to optimize the uptake by the plant and improve the NUE. For example, traditionally, the time of the application is decided by the farmer based on the observation of the leaf color. With new tools, like the chlorophyll meter or optical sensors, the evolution of the plant status can be followed more efficiently, and the determination of the plant needs more accurate (Bijay-Sing et al., 2017). Using that technique over the traditional recommendations resulted in the increasing of NUE by 30-40% and by 7% of the grain yield (Dobermann et al., 2002). The recovery efficiency average in Asia is of 31%, while it is about 40% for a site-specific N management (Cassman et al., 2002).

## **3 Salinity**

“Salinization is the process of excessive accumulation of soluble salts in soils that adversely affects the environmental health, the crop productivity, and ultimately the farmer profitability” (Hussain et al., 2018). Soil salinity is the second major abiotic stress after drought and before soil alkalinity. Abiotic stresses, that should be more

frequent in the future with the climate change, are the most damaging factors for the growth rate and crop productivity in the world (Gao et al., 2007). A soil is categorized saline with an Electrical Conductivity (EC) higher than 4 dSm<sup>-1</sup> (Singh et al., 2009). The salinity is present in 20% of the world irrigated land and is reducing by 1-2% every year the arable land (FAO, 2002).

The soil salinization can occur from different sources of salt (mostly chloride and sulfate of sodium). The dominant ones are from the rock weathering and rainfall (low amounts of salt but accumulation over time). Wind-transported salt, seawater intrusion or salts from poor quality irrigation are also non-negligent sources. Three types of salinization processes have been investigated. The first one occurs with saline groundwater, the groundwater with solubilized salts is rising up by capillarity and with the evaporation of the water, the concentration in salt is increasing. The second process is due to the accumulation in the topsoil layers of salts from non-groundwater (from rainfall, weathering, aeolian deposits). Finally, the third process is associated with salts introduced by irrigation (Rengasamy, 2006; Hussain et al., 2018).

### **3.1 Effect of Salt Stress**

The effects of salt stress depend of the plant growth stage (Figure 4), the duration and intensity of the stress. It impacts the plant growth by the nutrient acquisition and transport, the carbon fixation and the grain formation. All these physiological processes are affected through osmotic stress, ion toxicity, nutrient disparities and the combination of them (Hussain et al., 2018).

The most salt sensitive stages in rice crops are the seedling emergence, the growth of transplanted seedlings and the reproductive stage (panicle initiation to fecundation) as it can be observed in the Figure 4 (Singh et al., 2009; Hussain et al., 2018).

During the vegetative phase, the NaCl toxicity can delay the germination by the decrease of the soil osmotic potential, inhibiting the imbibition process, altering the protein synthesis or damaging the embryo. The seedling biomass is reduced due to the high sodium contents (Zafar et al., 2015). The leaf growth, tillering, and therefore the dry weight, are severely reduced due to a low rate of cell elongation, the stomatal closure and the increase of leaf temperature caused by osmotic stress and the accumulation of Na<sup>+</sup> in the shoot. In addition, if the stress is severe, leaf senescence, abscission and finally death can be observed (Hairmansis et al., 2014).

The other highly sensitive phase to salt stress is the reproductive one. The salt can induce a delay in the heading and sterility during the anthesis or fecundation.

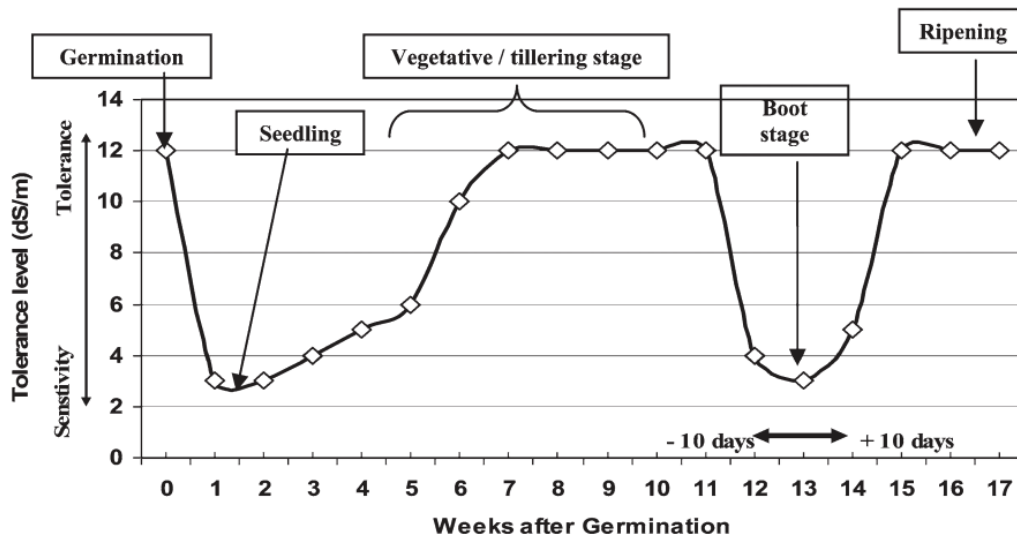


Figure 4: Salt tolerance evolution of the rice growth stages (Singh et al., 2009)

The roots are the first structure of the plant submitted to the saline conditions. However, the stress effects are less important than in shoots, due to the lower salt concentrations in the roots and to their capacity to export the toxic ions to the soil or shoots (Tester and Davenport, 2003). The salinity reduces the root length due a growth inhibition by a decreasing level of gibberellic acid (GA) (Wen et al., 2010). However, structural changes have been observed in root tip cells to adapt (thickening of the cell wall epidermis) and limit the absorption of toxic ions in rice (Lutts et al., 1996a). But Rahman has shown that an accumulation of sodium ions in vacuoles can also be a response of rice (Rahman et al., 2001).

The mineral uptake and assimilation in rice under saline condition is perturbed. A high concentration of NaCl causes nutritional disparities with a competition for the uptake of other ions and nutrients ( $\text{NO}_3^-$ , P, K, Mg, Ca, Mn...) (Lutts et al., 1996a; Hasegawa et al., 2000; Hussain et al., 2018). This disparity of nutrients and the high levels of  $\text{Na}^+$  are decisive. Indeed, the main metabolic toxicity of  $\text{Na}^+$  is caused by its competition with  $\text{K}^+$  and its replacement on usually  $\text{K}^+$  binding sites to activate enzymes and express cellular processes (Tester and Davenport, 2003). The  $\text{Na}^+/\text{K}^+$  ratio has a correlation with the salt tolerance. A low ratio is associated to tolerant varieties and a high one to sensitive rice (Gao et al., 2007). A problem in rice is the bypass flow, an apoplastic water flow inducing a leakage of sodium in the plant. This leakage is 10 times greater in rice than in wheat (Garcia et al., 1997; Tester and Davenport, 2003).

Photosynthesis is also affected, through the carbon fixation and the light utilization, by the salt stress. It has been observed that the leaf senescence is accelerated with salinity. Indeed, the NaCl decreases the chlorophyll and protein contents and increases the membrane permeability and the chlorophyll fluorescence (Lutts & al., 1996b). In response to salinity, stomatal perturbations by the osmotic stress and the high level of abscisic acid (ABA) directly influence the cell  $\text{CO}_2$  concentrations and the carbon fixation (Senguttuvel et al., 2014). In response, the increasing concentration of

Reactive Oxygen Species (ROS), which damages the cell, is problematic and the cell has to be detoxified (Zhu, 2001; Hussain et al., 2018).

### **3.2 Tolerance Mechanisms**

Several mechanisms are established by plants to tolerate a salt stress depending on its the duration and severity. The first response is the tolerance to osmotic stress, then comes the ion homeostasis and the tolerance at the whole plant level (Tester and Davenport, 2003; Hussain et al., 2018).

The tolerance process to the osmotic stress induces the compartmentalization of the cations (sodium and potassium), the production of stress proteins and compatible osmolytes (soluble sugars, proline, polyols). The objective is mainly to osmoregulate the cells potential (Lutts et al., 1999; Tester and Davenport, 2003), but also to detoxify the plants from the ROS or prevent their damages to the cellular structures (Zhu, 2001). Osmoregulation limits the stomatal closure and preserves the cell extension depending of the stress severity. This mechanism allows a reduced continuation of the rice growth and the production of new leaves.

Ion homeostasis is regulated by specific transporters in order to avoid a salt stress. Different processes can be done to achieve the homeostasis. First of all, the sodium influx can be controlled and excluded from the cell with  $\text{Na}^+/\text{H}^+$  antiporters on the plasma membrane. Secondly, the sodium can be compartmented into vacuoles with another type of  $\text{Na}^+/\text{H}^+$  antiporters on the tonoplast (Zhu, 2001). According to Raven (1985), osmotic adjustment by ions regulations are energetically better for the plant than with osmolytes synthesis, as the ions transport is less costly than the production.

The adaptation to the salinity can also be made at the plant level. Many strategies can be made. A first point is the uptake and distribution of  $\text{Na}^+$  within different parts of the plant. The sodium can therefore preferentially accumulate in the old leaves to preserve the new ones. Another extrusion pathway of the shoot salt is by hydathodes. Coupled to water transpiration release, salt can be extruded from the leaf blade. (Tester and Davenport, 2003; Wu, 2018).

## **4 Interaction Between NUE and Salt Stress**

Interaction between nitrogen use efficiency and salt stress tolerance in plants are various and complex but could be very promising to mitigate the stress. On the one hand, as it has been shown in section 2 (nitrogen), N is an essential nutrient for the plant growth and for the efficiency of its metabolism. On the other hand, salinity disturbs the

ions uptake, like N, and translocation with its antagonist effect, resulting in the creation of a nutrient imbalance inside the plant and a dysfunction of the metabolism.

While the overall interactions are not fully known, many studies have been made on the subject that nitrogen is contributing to salt tolerance (Ashraf et al., 2018). For example, the N role is crucial under salt stress by antagonism to NaCl, regulating the stomatal conductance (Tester and Davenport, 2003), by being a constituent of several compounds (proline, glycine betaine, glycophyte) adjusting the osmotic potential (Lutts et al., 1999; Tester and Davenport, 2003), having antioxidant activities (Zhu, 2001) and many more. The response to the stress is also dependent of the N form, the plant species, the salt concentration and the N rate application (Ashraf et al., 2018; Nhung et al., 2017).

Moreover, research conducted by Abdelgadir et al. (2005), based on different levels of nitrogen and salinity, has highlighted that an increase of the nitrogen concentration is beneficial for the plant development such as the shoot dry mass until a moderate salt level. However, under high salt level, the variation of N has no effect and the plant is just responding to the salinity. Additionally, the increase of N causes the reduction of the roots dry mass under salt stress. Finally, Nhung et al. (2017) have shown that the rice performs better on a low N concentration than on a high one under saline conditions due to a low demand of N by the plant.

## Chapter II: Interest and Objectives

The **main objective** of this thesis is to analyze the influence of rice (*Oryza sativa*) genotype over its Nitrogen Use Efficiency (NUE) under salinity stress. NUE and salinity stress are connected to each other as it has been shown in the previous chapter. The natural selection and breeding programs have led to plants more responsive to nitrogen supply and more tolerant to abiotic stress such as salinity. Nevertheless, the identification and a better comprehension of those mechanisms would improve significantly the variety selection and the fertilization management.

The **first specific objective** is to study trends, highlighted by the experiments, resulting from the combinations of salt-tolerant or sensitive cultivars, increasing nitrogen supplies and salt levels. This study will be conducted with an ANOVA analysis of many morphological and physiological measurements related to the three factors. The data collected are - among others - the dry weight, number of tillers, chlorophyll content, protein content, shoot minerals content, evapotranspiration and the tolerance to salinity.

The **second specific objective** is to attempt to understand the physiological patterns and plant responses associated with those trends. The specific experimental conditions, the analysis of the results and the associated literature could lead to the consideration of several hypotheses explaining the salt tolerance or sensitivity.

The global interest of this work fits in the development of a more sustainable nitrogen management for rice production under saline conditions. An adequate management could possibly contribute to maintain the cultivated area threatened by salinity under production. This interest goes through a better comprehension of NUE of various genotypes.



## **Chapter III: Materials and Methods**

To facilitate the reading and comprehension, this chapter is divided in two parts corresponding to different types of experimentations. The first part is composed of two experiments in hydroponics. The first one was a prospective study to observe the interactions of the nitrogen and salt treatment at the germination stage of rice. The second one was a consolidated experiment to study the above interactions during the vegetative stage, especially on the seedlings and the early tillering. The second part, or third experiment, was conducted in pots in order to validate the trends observed in the two other experiments in a controlled environment closer to the field conditions.

The first two experiments were conducted during October and November 2017 in hydroponics, in the phytotron of the laboratory of Ecophysiology and Plants Genetics for Sustainable Agriculture (ECAV) which is part of the Earth and Life Institute - Agronomy (ELI-A), Université catholique de Louvain (UCL), Belgium.

The third experiment took place during the 2018 spring season with pots inside a greenhouse at the Agronomy faculty of the Vietnam National University of Agriculture (VNUA) in collaboration with the ECAV laboratory.

This protocol is similar to the work of Adrien Heymans for his master thesis in 2017 on the same subject and is corresponding to an adapted replication of his experimentation.

### **1 Part 1: Evaluation of the Interactions between N, NaCl and Genotype in Hydroponics at UCL**

The objective of the following experiments in hydroponics was to characterize the interactions of the studied fixed parameters during the vegetative stage (seedling and early tillering): cultivars, nitrogen and salt concentrations, under strictly controlled conditions in order to limit unstable environmental effects and therefore allowing to focus more accurately on the plant responses.

For this part, the material of the two experiments has a common base, so the description of the treatments will be detailed in a first section.

## 1.1 Material and Experimental Conditions

### 1.1.1. Plant Materials

Three rice (*Oryza sativa* L. spp *indica*) varieties were selected for the experiments on the basis of their different response to salinity. All the cultivars are considered traditional. Two came from coastal areas of North Vietnam and an IRRI referential salinity tolerant from India was added. The table 1 below summarize the main characteristics.

Table 1: Rice varieties selected for the hydroponics experiments

	Salt tolerant	Salt sensitive
Trad. from North Vietnam	<i>Cuom</i>	<i>Ngoi</i>
Trad. from India	<i>Pokkali IRRI</i>	

Pokkali seeds were provided by the Plant Physiology Research Group laboratory (Groupe de recherche en physiologie végétale, GRPV, ELI-A, UCL) and the two other genotypes by VNUA (Vietnam).

Both of the studied Vietnamese rices, Cuom (salt tolerant) and Ngoi (salt sensitive) have a low yield and are relatively tall. They aren't currently being cultivated despite an acceptable production for Cuom in case of severe salinity.

Pokkali was selected in order to make a comparison of the Vietnamese varieties with a word reference for the salinity tolerance. Originated from a coastal region of India (Kerala), it's a tall cultivar (165-176 cm), photoperiod-sensitive, susceptible to lodging, with long, broad, dark and droopy leaves. The flowering occurs quickly (72-104 days) but the leaves senesce also quickly after it. It produces vigorous seedlings but has a low tillering and above all a low yield capacity. In addition, the grain, with a red pericarp, has a poor cooking quality (Gregorio et al., 2002). This genotype is a good parent for breeding and selection of new tolerant cultivars to salinity (Gregorio et al., 1993). Many somaclonal variants were created in order to have a qualitative improvement on Pokkali characteristics. Those variants had the effect of creating a subcluster of Pokkali varieties in *Oryza sativa* L. spp. *indica* (Rahman et al., 2016).

### 1.1.2. Experimental Conditions

The experiments were conducted in a phytotron in UCL during October and November 2017. The parameters below were fixed to ensure stable conditions.

- The photoperiod day length was of 12 hours
- The day/night temperature was 30 °C/25 °C
- The relative humidity was 85-95%
- The light intensity was set at 210  $\mu\text{mol}/\text{m}^2\text{s}$

The rice was cultivated in hydroponics based on the Yoshida standard solution (Yoshida, 1976) with an exception for the Nitrogen and NaCl concentrations, which will be detailed in subsection 1.1.3. The solution was changed every week and the pH was adjusted daily to 5.0-5.5 by using 2M KOH or 1M HCl.

### 1.1.3. Treatments

The six treatments were composed by a combination of two Nitrogen concentrations and three salt levels (Table 2). The Nitrogen concentrations were composed from ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) by a dilution of 1 and 8 times of the Yoshida standard (2.86 mM N) in the nutrient solution. The salt levels were obtained with a dilution of Sodium chloride (NaCl).

Table 2: Nitrogen and salt concentrations applied as treatments in hydroponics

Levels of Nitrogen concentration applied	<i>N Standard</i>		<i>1/8 N</i>
	N [mM]	2,86	
Levels of salt conditions	No salt condition	Moderate salt condition	Strong salt condition
	NaCl [mM]	0	56,5
Equivalent in EC [dS/m]	0	6	11,5

## 1.2 Prospective Study at the Germination Stage

### 1.2.1. Experimental Specificities

The plants were directly submitted to the treatments at the sowing and the sampling was made at the three expanded leaves stage that occurred during the second week of cultivation.

For this experiment, the observations focused on the Cuom and Ngoi cultivars (the available seeds for Pokkali weren't sufficient to make conclusive analysis). Each treatment was applied to 40 seeds of each cultivar with 1L of the solution in a container of 1.5L. The seeds were put in 2x4 holes separated by 2 cm (5 seeds/hole) of an extruded polystyrene frame, with a net below to maintain the seeds, floating on the solution. The containers were rearranged every 3 days.

To sum up, we had 6 treatments distributed in 12 containers, which had 40 repetitions of the 2 genotypes, for a total number of 480 plants:

$$480 \text{ plants} = 3 \text{ NaCl} \times 2N \times 2 \text{ genotypes} \times 40 \text{ repetitions}$$

### 1.2.2. Data Collection

The plants were sampled when the three-leaf stage was observed. The sampling was made of 20 repetitions (20 plants) per genotype and treatment. The shoot and roots were separated at the crown to make the data collection.

The data collection was composed of the following morphological measurements:

- Shoot and root system length (cm) for an estimation of the growth rate
- Fresh and dry weight (g) of the shoot and roots separately (by ten repetitions). The dry weight was measured after the passage of the plants in an oven at 70 °C for 96 hours in order to have a constant weight.
- Survival rate which is the percentage of the surviving rice plants at the time of sampling.
- The number of days after sowing to obtain the three-leaf stage for the sampling.

## 1.3 Evaluation during Vegetative Stage

### 1.3.1. Experimental Specificities

The calendar of the experiment is detailed in the following figure (Figure 5). Two weeks were set between each operation after the germination.



Figure 5: Calendar of the experiment on the vegetative stage in hydroponics

For this experiment, the plants were cultivated in 25L containers. Each container carried 40 plants (14 Cuom, 13 Ngoi and 13 Pokkali) randomly disposed in holes (5x8 holes separated by 2 cm) on an extruded polyester frame with a net floating on the solution. At the sowing, three seeds were put in each hole and one week later, the number of plants was thinned to one per hole. The disposition of the containers was changed every week randomly to avoid any disposition influence of the phytotron.

The total number of plants cultivated was 720. This corresponded to six treatments applied on three genotypes with 40 repetitions (40 plants) for each and disposed in 18 containers:

$$720 \text{ plants} = 3 \text{ NaCl} \times 2 \text{ N} \times 3 \text{ genotypes} \times 40 \text{ repetitions}$$

### 1.3.2. Sampling

A sampling time (two and four weeks of treatment) was composed of ten repetitions, which means ten plants, per genotype and treatment (18 possibilities).

The shoot and roots were separated at the crown to make the data collection. Five repetitions were frozen at -80 °C for further analysis on fresh material. The five other repetitions were collected to measure basic agronomical variables on a fresh matter basis. After those measurements, the samples were dried, as seen in the subsection 1.2.2. protocol.

For the second sampling time, the five dried repetitions were used to measure the nitrogen, carbon, potassium, chloride and sodium content. As for the frozen repetitions, three of them were collected to determine the proteins content and the two others, composed only of the leaves, were used to analyze the chlorophyll content.

### 1.3.3. Data Collection

A sampling time was composed of the following morphological measurements:

- Shoot and root system length (cm) for an estimation of the growth rate
- Fresh and dry weight (g) of the shoot and roots separately
- The numbers of root tips at 0.5 cm from the crown
- The numbers of tillers, including the main stem
- The overall estimation of the severity of the salt stressed symptoms. The estimation was based on the Standard Evaluation System - SES (IRRI, 1997), represented on the Table 3 below.

The following variables related to the photosynthetic function were recorded with an Infra-Red Gas Analyser (IRGA - LCI-SD) on the last fully expanded leaf:

- The net assimilation rate of CO<sub>2</sub> [ $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ]. The net assimilation rate of CO<sub>2</sub> is given by the mass balance of CO<sub>2</sub> in all gases including water vapor of the leaf.
- The Transpiration rate [ $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ]. The transpiration process is calculated through the mass balance of water vapor in an open system.
- The stomatal conductance [ $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ]. It measures the entering rate of the CO<sub>2</sub> in the stomata. It is a function of the stomata density, the stomata aperture and the size of the stomata.
- The intercellular CO<sub>2</sub> concentration [ $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ Air}$ ]

Table 3: Standard Evaluation System (SES) score of visual salt injury at the seedling stage for rice (IRRI, 1997).

Score	Observation	Classification
1	Normal growth, no leaf symptoms	highly tolerant
3	Nearly normal growth, but leaf tips or a few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongation	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible
9	Almost all plants dead or dying	High susceptible

Only for the second sampling time, the following physiological variables were measured:

- Chlorophyll contents of the leaves [ $\text{mg/g}$  of fresh matter]. The Lichtenthaler protocol (1987 - annex 1) was used to determine the concentrations of chlorophyll ( $\alpha$  and  $\beta$ ) and carotenoids (xanthophyll +  $\beta$ -carotene). The

absorbance of the samples was read at the three corresponding wavelengths (663.2, 646.8 and 470 nm, respectively) by a spectrophotometer (DU®R 640, Beckman Coulter, South Pasadan, USA).

- Total proteins content of the shoots and roots [mg/g of fresh matter]. The measurement was based on the Bradford method (annex 1) and the absorbance was read at 595 nm on a spectrophotometer (SPECTROstar Nano, BMG Labtech).
- Carbon and nitrogen content in shoots [mass N % of the dry matter]. The measures were taken with the Carbon-Nitrogen chromatography (vario EL cube).
- The NUE are calculated with the following equations:
  - absorbed NUE (aNUE):

$$aNUE [\%] = \frac{N \text{ absorbed by the plant } [g N]}{N \text{ applied } [g N]} \times 100$$

- physiological NUE (pNUE):

$$pNUE = \frac{\text{Total dry weight } [g]}{N \text{ absorbed by the plant } [g N]}$$

- agronomical NUE (agNUE)

$$agNUE = \frac{\text{Total dry weight} [g]}{N \text{ applied } [g N]}$$

- Na and K content of the shoots [mg/kg of dry matter]. It was measured (protocol in annex 1) with inductively coupled plasma atomic emission spectroscopy (ICP-AES) with iCAP 6500 device (Thermo Scientific).
- Chloride (Cl) content of the shoots [mg/kg of dry matter]. The protocol is detailed in annex 1 and the measurements were made on an ion chromatography system (Dionex ICS-2000).

## 1.4 Analyses of the Observations

In order to find principal effects and interactions between the fixed parameters, a statistical method was used to evaluate the regression model below (Equation 1) with the significance of the parameters and interactions. However, if a parameter or an interaction was non-significant in the model (complete model), the removal of it should be done and therefore to use a restrained model (Equation 2). The data collected as explained in the previous point were analyzed with this statistical tool. The complete generalized linear model (glm) tested for the variables is:

Equation 1: Complete generalized linear model

$$Y_i = \beta_0 + X_i NaCl + Z_i N + W_i Gen + X_i Z_i (NaCl \times N) + X_i W_i (NaCl \times Gen) + Z_i W_i (N \times Gen) + X_i Z_i W_i (N \times Gen \times NaCl) + \varepsilon_i$$

Where:

- Y represents the tested dependent variable on the model without interaction.
- N is the explicative variable of the nitrogen concentration applied.
- Gen is the explicative variable of the genotype.
- NaCl is the explicative variable of the salt condition of the hydroponic solution.
- X, Z, W are the observations of the explanatory variables.
- $\varepsilon_i$  is the residual error.

All parameters were treated as discrete values. To determine if the restrained model had to be used instead of the complete one, the following F-test was submitted:

H<sub>0</sub>:  $Y = X_1\beta_1 + \varepsilon_R$  as null hypothesis which corresponds to the Restrained model.

H<sub>1</sub>:  $Y = X_1\beta_1 + X_2\beta_2 + \varepsilon_C$  is the complete model after looking at the p-value given by the following equation:

$$F_{obs} = \frac{SSE_{Restrained} - SSE_{Completed} / Df_R - Df_C}{SSE_{Completed} / Df_C} \sim F_{p2, N-p}$$

With:

- SSE = Sum of Squared Errors of prediction
- Df = Degree of freedom

If the p-value was significant, which is corresponding to a result lower than 0.05 in order to have a 95% confidence interval, then the complete model was used. In the other case, the restrained model below, where the triple interaction was left out, was run:

Equation 2: Restrained generalized linear model

$$Y_i = \beta_0 + X_i NaCl + Z_i N + W_i Gen + X_i Z_i (NaCl \times N) + X_i W_i (NaCl \times Gen) + Z_i W_i (N \times Gen) + \varepsilon_i$$

With the selected model, an analysis of the variance was made (type III ANOVA) to determine effects of the parameters and their interactions. If a positive response to it was found, a contrast analysis with Tukey method and the least squares means were

calculated. Then a boxplot was established with the raw data to represent that tendency. As usual, if the P-value of the test was lower than 0.05, it was considered significant. In addition, the  $R^2$  was calculated to see the accuracy of the model used.

When significant results were obtained, the Least Squares MEANS (LSMEANS) were detailed by a table for the three primary parameters or represented by a graph of the interaction between the concerned parameters. Only the most conclusive trends were represented.

The matrix of Pearson was established in order to see the correlations between the sampled variables.

The statistical analysis was made with the Statistical Analysis Software (SAS, 9.4). The different scripts used are in annex 2.

## **2 Part 2: Validation of the Interactions between N, NaCl and Genotypes in Pots at VNUA, North Vietnam**

Some trends were highlighted with the experiments in hydroponics. As a finality of the research is to use those results in fields, a similar experiment has been conducted in Vietnam to confirm the trends in an environment close to field conditions.

The experiment was set during the spring rice season at VNUA in Hanoi, North Vietnam. The seeds were sown in the nursery two weeks before transplanting the plants individually in pots of 4 kg of flood clayey plain soil from the Red River (North Vietnam). The pots were maintained in waterlogged condition during the whole experimentation. The treatments were similar to the previous experiments with the salt conditions, but four nitrogen concentrations were applied to four cultivars (Cuom, Ngoi, FL478 and IR28). The experiment went through the harvest, but data collection was focused on the tillering stage. Two sampling times were made, the most important at the tillering stage and the second at the harvest.

The Red River delta area was appropriate for the experiment like it is one of the most productive agricultural areas from the North Vietnam, with 74% of agricultural land used for the rice cultivation (573 900 ha), producing one third of rice in Vietnam (GSO, 2012; Nguyen & al., 2016). But with the sea level rising and low availability of the Red River water during the dry season, deep saltwater intrusion in the delta fields is a preoccupation for the future. In addition, Vietnam is now an important rice exporting country with the highest rice yield among the tropical countries due to the agricultural intensification policy of the government (Takeuchi & al., 2018). A consequence is that the overuse of nitrogen fertilization can be present in this region to reach a high yield.

Thus, the challenges are multiple in that rice area, from dealing with salt intrusion and accumulation, to limiting and applying an adequate fertilization. For all those reasons, the choice of this location is suited for the study.

## 2.1 Plant Materials

The two traditional cultivars from Vietnam (Cuom Di and Ngoi) were used for the experiment and two others improved varieties were added (FL478 and IR28) as we can see in the Table 4 below.

Table 4: Rice varieties selected for the Vietnamese experiment

	Salt tolerant	Salt sensitive
Trad. from North Vietnam	<i>Cuom</i>	<i>Ngoi</i>
Improved by IRRI	<i>FL478</i>	<i>IR28</i>

The two improved genotypes have a high yield capacity with a heavy panicle. They are shorter than the traditional and so less prone to lodging. FL478 is a salt tolerant recombinant inbred by IRRI from a cross between Pokkali and IR29. In response to salinity, the shoot ratio  $\text{Na}^+/\text{K}^+$  is kept lower and the carbohydrate contents higher than for the salt sensitive like IR28 (Vijayalakshmi, 2014). From a QTL analysis, a region named Saltol on the chromosome 1 has been highlighted for salinity tolerance (Thomson & al, 2010). The IR28 cultivar is a salt-sensitive rice developed by IRRI in order to have a reference for this sensitivity.

Pokkali couldn't be used in this experiment because it was not adapted to the environment conditions of the spring season in Vietnam. Indeed, the cultivar is photoperiodic dependent, and the daylength was not appropriate at this period. Nevertheless, Pokkali can be cultivated during the summer season in the North of Vietnam.

Three sampling times of respectively 6, 3 and 3 repetitions were made, the total number of plants was:

$$576 \text{ plants} = 3 \text{ NaCl} \times 4 \text{ N} \times 4 \text{ genotypes} \times 12 \text{ repetitions}$$

## 2.2 Experimental Conditions

The experiment took place in a nethouse at the experimental crop facility of the Vietnam National University of Agriculture (VNUA), Gia Lam District, Hanoi, Vietnam. The seeds were sown in a nursery field the 26<sup>th</sup> of February and transplanted the 10<sup>th</sup> of March, at the three-leaf stage, in pots for the beginning of the treatments. The experiment finished the 29<sup>th</sup> of June. The schedule is shown on Figure 6.

The alluvial soil used in the pots, freshly taken from the Red River delta, had been dried before weighing 4 kg for each pot. The plants were always kept in waterlogged conditions with a control of the conductivity for the treatment stability. The influence of the position was limited by the weekly randomization of the pots. The dimensions of a pot were a diameter of 20 cm with a depth of 20 cm (similarly to the depth of the top layer in paddy fields).



Figure 6: Schedule of the Vietnamese experiment

### 2.2.1. Fertilization

The amounts of P and K fertilizers were each of 0.5g pot<sup>-1</sup>. The quantity of nitrogen supply, depending on the treatment will be explained in the following subsection. The N-form used was urea while K<sub>2</sub>O was the source of K, and P<sub>2</sub>O<sub>5</sub> for P fertilizer. Supplement fertilizer (10 g pot<sup>-1</sup>) was given as well, containing humic acid (2.5%), Ca (1.0%), Mg (0.5%), S (0.3%), and a handful of bacteria such as *Aspergillus* (1.10<sup>6</sup> CFU g<sup>-1</sup>), *Azotobacter* (1.10<sup>6</sup> CFU/g), and *Bacillus* (1.10<sup>6</sup> CFU g<sup>-1</sup>).

The fertilization process was split in three times to avoid losses of N and K and to optimize the uptake by the plants. The times of application were before transplanting for the basal dressing, ten days after transplanting for the first top-dressing, and two weeks before heading for the second top-dressing. The nitrogen rates were respectively of 20%, 50% and 30% for those dressings. In the same way, the potassium rates were respectively of 20%, 30% and 50%. At transplanting, the first input of fertilizers was applied directly in the water and mixed with the soil. Other top-dressing applications were added into the surface water and dissolved.

### 2.2.2. Treatments

Similar treatments than the ones chosen in Belgium were applied with three salinity levels and four nitrogen concentrations (Table 5). Three salt conditions were controlled by diluting or increasing the saltwater concentration in pots with a concentrated NaCl solution (200g NaCl L<sup>-1</sup>). The salinity was controlled by the conductivity (EC) every three days with a conductivity meter on the water surface layer. The standard nitrogen concentration was of 1g per pot (added on urea form, CO(NH<sub>2</sub>)<sub>2</sub>), which was the equivalent of standard 320Kg N ha<sup>-1</sup>.

Table 5: Nitrogen and salt concentrations applied as treatments in Vietnam

<b>Levels of Nitrogen concentration applied</b>	<i>N Standard</i>	<i>1/2 N</i>	<i>1/4 N</i>	<i>1/8 N</i>
N [g]	1 ± 0.001	0.5 ± 0.001	0.25 ± 0.001	0.125 ± 0.001
Approximate equivalent in [N Kg ha <sup>-1</sup> ]	320 ± 0.32	160 ± 0.32	80 ± 0.32	40 ± 0.32
<b>Levels of salt conditions</b>	No salt condition	Moderate salt condition	Strong salt condition	
EC [dS m <sup>-1</sup> ]	0 ± 0.5	4 ± 0.5	8 ± 0.5	

## 2.3 Sampling

The main sampling was made during active tillering on the 18<sup>th</sup> of April 2018. Six repetitions were collected, and the shoot was separated from the roots at the crown to make the measurements. Another sampling has been made on three repetitions at the heading stage between the 16<sup>th</sup> and the 25<sup>th</sup> of May. Finally, the last sampling was made at the harvest between the 22<sup>nd</sup> and the 29<sup>th</sup> of June. After all the measurements on the fresh matter, the plants were dried for 3 days at 80 °C in order to weight them and to conserve the material before sending the samples to UCL for further analysis. Unfortunately, we were not able to analyze the nitrogen and carbon content in time to compare them to the results from the hydroponics.

### 2.3.1. Data Collection

The data collected during the first sampling at active tillering was composed of the following measurements:

- The fresh and dry weight [g] of the shoot and roots. The dry weight was obtained as explained in the subsection 2.3.
- The agronomical NUE defined by:

$$agNUE = \frac{\text{Total dry weight [g]}}{\text{N applied [g N]}}$$

- The photosynthetic functions. The variables were the net assimilation rate of CO<sub>2</sub> [ $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ], the transpiration rate [ $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ], the stomatal conductance [ $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ], and the intracellular concentration of CO<sub>2</sub> [ $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ Air}$ ]. They were measured with a gas analyzer and fluorescence system (Licor 6400XT) on the last fully expanded leaf. For the data collection of those functions, the light intensity had to be high, so the sampling was delayed on the 8<sup>th</sup> of May (still during active tillering) due to the bad weather.

The following variables were collected every week until the destructive measurement at active tillering, or when the plant was considered dead with the SES scale:

- The plant height [cm]
- The number of tillers
- The Soil-Plant Analysis Development (SPAD): the measurements were made on the last fully expanded leaf on the two thirds of the leaf length with a chlorophyll meter (SPAD 502Plus).

During the heading stage sampling on three repetitions (on the surviving plants), the following parameters were collected:

- The fresh and dry weight [g] of the shoot and roots.
- The agronomical NUE defined above.

During the harvest sampling on three repetitions (on the surviving plants), the following parameters were collected:

- The fresh and dry weight [g] of the shoot and roots
- The grain yields: the number of filled grain per plant, the weight of filled grain per plant and the number of panicles per plant were measured.
- The agronomical NUE defined above.

## 2.4 Analysis of the Observations

A similar statistical analysis was made as for the experiment in hydroponics (subsection 1.4). But we had to consider the variability and the absence of data for the most severe salt conditions, resulting in an incomplete set of data and the difficulty to analyze them correctly. For the photosynthetic variables, the strong salinity had to be put aside in order to conduct the ANOVA analysis.

## **Chapter IV: Results**

This chapter is divided in two sections. The first section presents the results of the two experiments in hydroponics (Belgium), while the second section deals with the experiment in Vietnam. Inside the sections, the morphological variables were presented in first place and then the physiological variables in second place. The interactions between the fixed parameters (genotype, NaCl and nitrogen) and the variables measured were highlighted with the statistical ANOVA analysis.

The results were presented separately with an analysis based on the statistics for each parameter and interaction. To illustrate the results and their trends, figures of the significant interactions effects were displayed. To reduce the number of figures, the graphs without any additional information to the presented ones were rejected.

### **1 Experiments in Hydroponics**

#### **1.1 Experiment during Germination**

The number of repetitions was too low to establish a statistical analysis on the variables excepted for the shoot length. Therefore, only one ANOVA analysis has been realized on the shoot length. The other variables followed the trends of the shoot length. The analysis concerned only morphological variables.

The survival rate was the same independently from the cultivar and N concentration. It was modified by the salinity levels with a survival rate of 99% for the no salt condition, 94% for the medium salinity and 49% for the strong salinity. Excepting a few seeds, the germination occurred for the totality of the treatments. The salt sensitivity and the possible death were observed on the seedlings after emergence.

The number of days necessary for the sampling at the three-leaf stage can be observed in the Table 6 below. The low nitrogen concentration reduced the plant growth for Cuom under the control and the medium salinity. However, the N concentration did not seem to have any influence under the strong salinity. The increasing salinity seems to delay the growth rate especially under the strong salinity. Those results need to be taken cautiously as a complementary information due to the low number of repetitions realized and cultivars tested.

Table 6: Number of days to attain the three-leaf stage sampling

<b>Genotype</b>	<b>Salinity [dS/m]</b>	<b>N conc. [mM]</b>	<b>Number of days for the three-leaf stage</b>
<b>Cuom</b>	0	2,86	11
		0,358	14
	6	2,86	11
		0,358	14
	11,5	2,86	14
		0,358	14
<b>Ngoi</b>	0	2,86	10
		0,358	10
	6	2,86	11
		0,358	11
	11,5	2,86	14
		0,358	14

### 1.1.1. Shoot Length

The ANOVA (Table 7) of the complete model showed that the triple interaction parameter was not significant on the shoot dry weight, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2= 0.90$ ). The effects of genotype, NaCl and the interaction genotype-N on the shoot length were significant.

Table 7: ANOVA models of the shoot length at the three leaves stage

<i>Type III ANOVA - Shoot length</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.4856	0.3532	<.0001	0.2534	0.8293
Pr > F	<.0001	<.0001	0.4496	0.3211	<.0001	0.2619	

**Genotype influence:** The genotype has a significant effect on the shoot length (Table 8). Cuom was significantly higher than Ngoi.

Table 8: LSMEANS of the shoot length in function of the genotype at the three leaves stage

	<i>LSMEANS - Shoot length [cm]</i>
<b>Cuom</b>	26.98 ± 0.49
<b>Ngoi</b>	16.05 ± 0.47

**NaCl influence:** The salt had a significant negative influence on the shoot length. The means length of the medium salt level and strong salt level corresponded to about 80% and 63% of the control, respectively.

**Interaction genotype-N:** The interaction had a significant effect on the shoot length. All the combinations were significantly different from each other, but the differences were very tiny and only the genotype influence was noticeable (Figure 7).

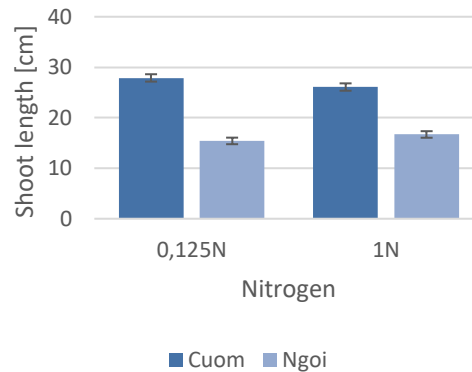


Figure 7: Shoot length at the three-leaf stage in function of the nitrogen concentration for the 2 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

## 1.2 Experiment during the Vegetative Stage

Due to the abundance of data, the results presented in this section were focused on the sampling at 4 weeks of treatment. The effects of this sampling were more significant than the one at 2 weeks of treatment. Moreover, the sampling at 4 weeks had the closest conditions to the active tillering sampling ones for the Vietnamese experiment and was thereby the most pertinent for comparison.

### 1.2.1. Morphological Adjustment

#### 1.2.1.1. SES

The ANOVA (Table 9) of the complete model showed that the triple interaction parameter was not significant on the SES, therefore the restrained model was calculated

and used ( $R^2= 0.70$ ). The effects of many parameters were significant on SES. The interactions N-NaCl and genotype-N were not significant.

Table 9: ANOVA models of the SES at 4 weeks of treatment

<i>Type III ANOVA - SES</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	0.0510	0.1428	<.0001	0.1036
Pr > F	<.0001	<.0001	<.0001	0.0585	0.1566	<.0001	

**Genotype influence:** Under the strong salt conditions, the genotype had a significant influence on SES (Table 10). The salt-tolerant varieties had significantly lower SES than the salt-sensitive (Ngoi). However, Cuom SES was higher than Pokkali SES, which is a salt-tolerant cultivar reference.

Table 10: SES values in strong salt conditions at 4 weeks of treatment. The complete model was used to evaluate the SES LSMEANS

	<i>SES Values</i>
<b>Cuom</b>	3 ± 0.67
<b>Pokkali</b>	4.4 ± 0.67
<b>Ngoi</b>	6.6 ± 0.67

### 1.2.1.2. Shoot Dry Weight

The ANOVA (Table 11) of the complete model showed that the triple interaction parameter was not significant on the shoot dry weight, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2= 0.85$ ). Most of the parameters were significant on the shoot dry weight. Only the interaction genotype-N was not significant. The correlation between shoot dry weight and the total dry weight was high (90%). Thus, the results of the total dry plant were not presented in order to avoid repetitions.

Table 11: ANOVA models of the shoot dry weight at 4 weeks of treatment

<i>Type III ANOVA - Shoot dry weight</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	0.0003	0.9252	0.0048	0.9452
Pr > F	<.0001	<.0001	<.0001	0.0002	0.9220	0.0036	

**Genotype influence:** The genotype has a significant effect on the shoot dry weight as it can be observed in the Table 12 and the Figure 8b. Cuom was significantly over the two other cultivars which were not different from each other.

Table 12: LSMEANS of the shoot dry weight in function of the genotype at 4 weeks of treatment

	<i>LSMEANS - Shoot dry weight [g]</i>
<b>Cuom</b>	2.00 ± 0.13
<b>Pokkali</b>	0.71 ± 0.13
<b>Ngoi</b>	0.81 ± 0.13

**NaCl influence:** The salt had a significant negative influence over the shoot dry weight (Figure 8). The means dry weights of the medium salt level and strong salt level corresponded to about 59% and 39% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the shoot dry weight (Figure 8a). The mean dry weight of the 0.125 standard N concentration corresponded to 71% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on the shoot dry weight and followed the trends observed (the positive effect of N and the negative of NaCl) but they were attenuated for the medium and mostly the strong NaCl level (Figure 8a).

**Interaction genotype-NaCl:** The interaction had a significant effect on the shoot dry weight. As it can be observed in the Figure 8b, the difference was significant for Cuom over the other cultivars, but those last were not to each other. Cuom was really higher than the others and especially under saline conditions.

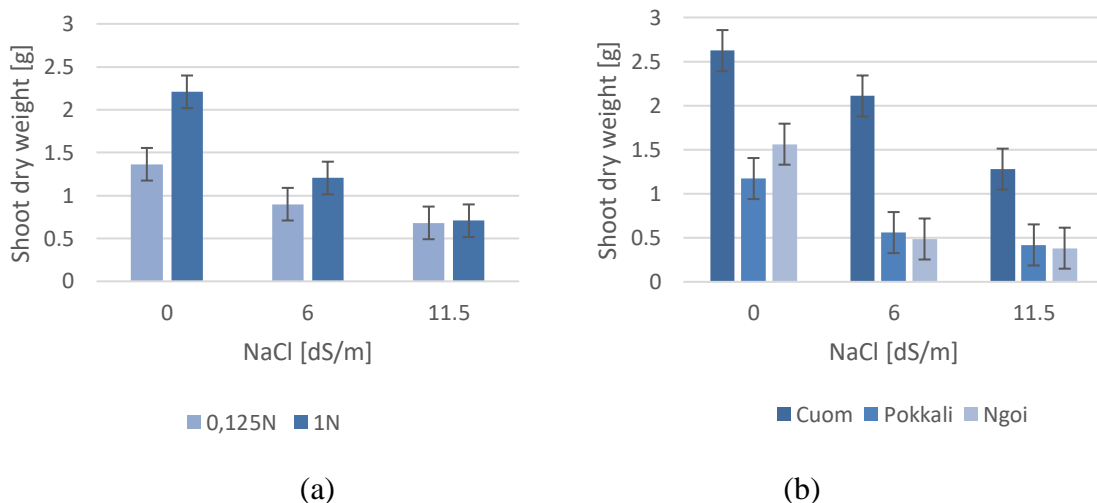


Figure 8: Shoot dry weight at 4 weeks of treatment: (a) in function of the salt concentration for the 2 nitrogen concentrations; (b) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.1.3. Root Dry Weight

The ANOVA (Table 13) of the complete model showed that the triple interaction parameter was not significant on the root dry weight, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2 = 0.84$ ). Most of the parameters were significant on the root dry weight. Only the interaction N-NaCl was not significant.

Table 13: ANOVA models of the root dry weight at 4 weeks of treatment

<i>Type III ANOVA - Root dry weight</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.0136	0.2673	0.0004	<.0001	0.2621
Pr > F	<.0001	<.0001	0.0143	0.2732	0.0004	<.0001	

**N influence:** The nitrogen had a significant negative effect on the root dry weight, in opposition with the positive influence on the shoot dry weight (Table 14 and Figure 9). The mean dry root weight of the 0.125 standard N concentration corresponded to 126% of the standard nitrogen one.

Table 14: LSMEANS of the root dry weight in function of N concentration at 4 weeks of treatment

	<i>LSMEANS - Root dry weight [g]</i>
<b>0.125N</b>	0.19 ± 0.02
<b>1N</b>	0.15 ± 0.02

**Interaction Genotype-N:** The effect of the interaction was significant on the root dry weight (Figure 9). The effects can be observed mainly with the genotype influence, Cuom was significantly really higher than the other cultivars with the negative effect of N on Cuom (insignificant for the other cultivars).

**The other parameters:** The effects were significant on the dry root weight and similar to the observations concerning the shoots (subsection 1.1.1.).

### 1.2.1.4. Shoot/Root Dry Weight Ratio

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the shoot/root dry weight (Pr > F: 0.3657), therefore the restrained

model was calculated. The restrained model was less suitable for analysis ( $R^2= 0.65$ ) and the fit diagnostics were not so conclusive.

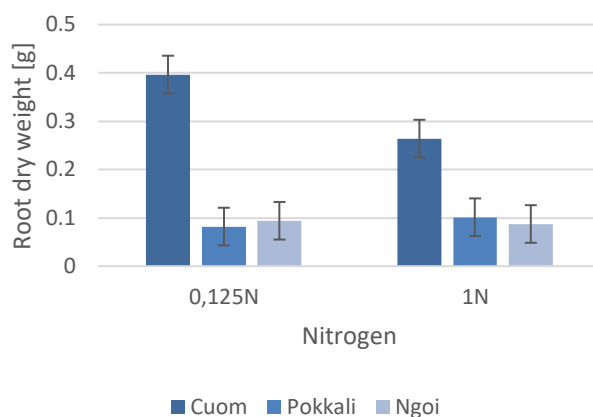


Figure 9: Root dry weight at 4 weeks of treatment in function of the N concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.1.5. Number of tillers

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the number of tillers ( $Pr > F: 0.8980$ ), therefore the restrained model was calculated. The restrained model was not suitable for analysis ( $R^2= 0.68$ ) and the fit diagnostics were not conclusive.

### 1.2.1.6. Shoot Length

The ANOVA (Table 15) of the complete model showed that the triple interaction parameter was not significant on the shoot length, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2= 0.93$ ). Most of the parameters were significant on the shoot length. Only the interaction genotype-N was not significant.

Table 15: ANOVA models of the shoot length at 4 weeks of treatment

<i>Type III ANOVA - Shoot length</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	0.0008	0.5618	<.0001	0.8086
Pr > F	<.0001	<.0001	<.0001	0.0006	0.5512	<.0001	

**Genotype influence:** The genotype had a significant influence on the shoot length. The cultivars were significantly different from each other (

Table 16 and Figure 10).

Table 16: LSMEANS of the shoot length in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Shoot length [cm]</i>
<b>Cuom</b>	99.9 ± 2.6
<b>Pokkali</b>	76.5 ± 2.6
<b>Ngoi</b>	66.3 ± 2.6

**N influence:** The nitrogen had a significant positive effect on the shoot length (Figure 10a). The mean shoot length of the 0.125 standard N concentration corresponded to 86% of the standard nitrogen one.

**Interaction N-NaCl:** The effect of the interaction was significant, and all the results were different from each other excepted the two strong salt levels (Figure 10a). The difference between the N concentration (positive effect of N) was attenuated by the increasing salinity.

**Interaction genotype-NaCl:** The effect of the interaction was significant on the shoot length. All the results were significantly different for each salt level excepted for Pokkali and Ngoi at the strong salt one (Figure 10b). The difference between Cuom and the other cultivars was increasing with the salt stress (with Cuom having the highest length).

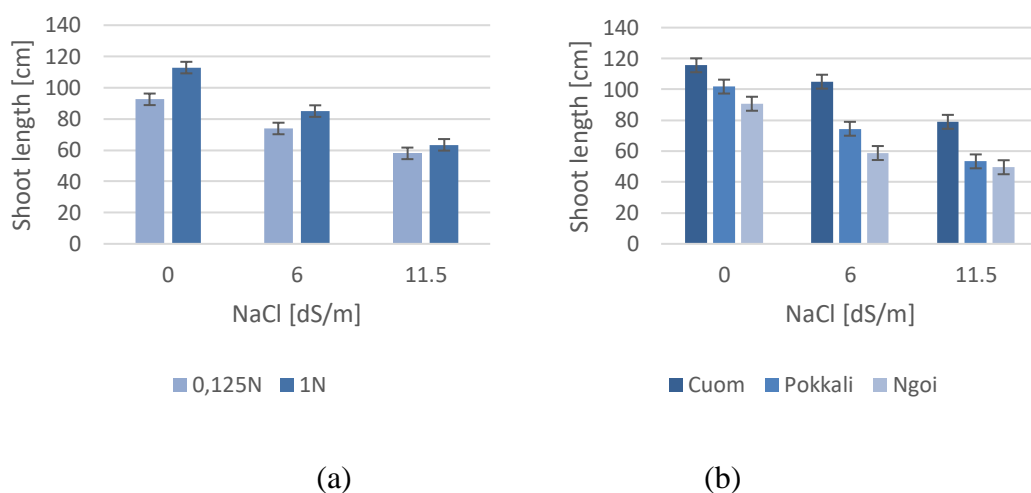


Figure 10: Shoot length at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the salt concentration for the 3 genotypes.

The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval

of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.1.7. Root Length

The ANOVA (Table 17) of the complete model showed that the triple interaction parameter was significant on the root length. The complete model was suitable for analysis ( $R^2 = 0.90$ ). All the parameters were significant on the root length.

Table 17: ANOVA complete model of the root length at 4 weeks of treatment

<i>Type III ANOVA - Root length</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	<.0001	0.0014	0.0031	0.0118

**Genotype influence:** The genotype had a significant influence on the root length. Cuom was significantly different from the other cultivars (Table 18).

Table 18: LSMEANS of the root length in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Root length [cm]</i>
<b>Cuom</b>	37.7 ± 1.7
<b>Pokkali</b>	15.9 ± 1.7
<b>Ngoi</b>	17.1 ± 1.7

**NaCl influence:** The salt had a significant negative influence on the root length (Figure 11). The means shoot length of the medium salt level and strong salt level corresponded to about 71% and 68% of the control, respectively. Only the control was significantly different from the medium and strong salt levels.

**N influence:** The nitrogen had a significant negative effect on the root length (Figure 11). The mean shoot length of the 0.125 standard N concentration corresponded to 135% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant negative effect on the root length (Figure 11a). The effect of the increasing N was negatively significant on the control, the difference was still present for the medium salinity but strongly attenuated (no difference under strong salinity). The root length stayed constant for the standard N.

**Interaction genotype-N:** The effect of the interaction was significant on the root length. Cuom was higher than the other cultivars, with a higher difference under the low N

concentration. The negative nitrogen effects can be observed mainly for Cuom and Ngoi (Figure 11b).

**Interaction genotype-NaCl:** The interaction had a significant effect on the root length. Cuom had a higher root length than the other cultivars. However, Cuom was affected continuously by the negative influence of NaCl, while Pokkali and Ngoi had a negative effect between the control and the medium but stayed constant under the medium and strong salinity (Figure 11c).

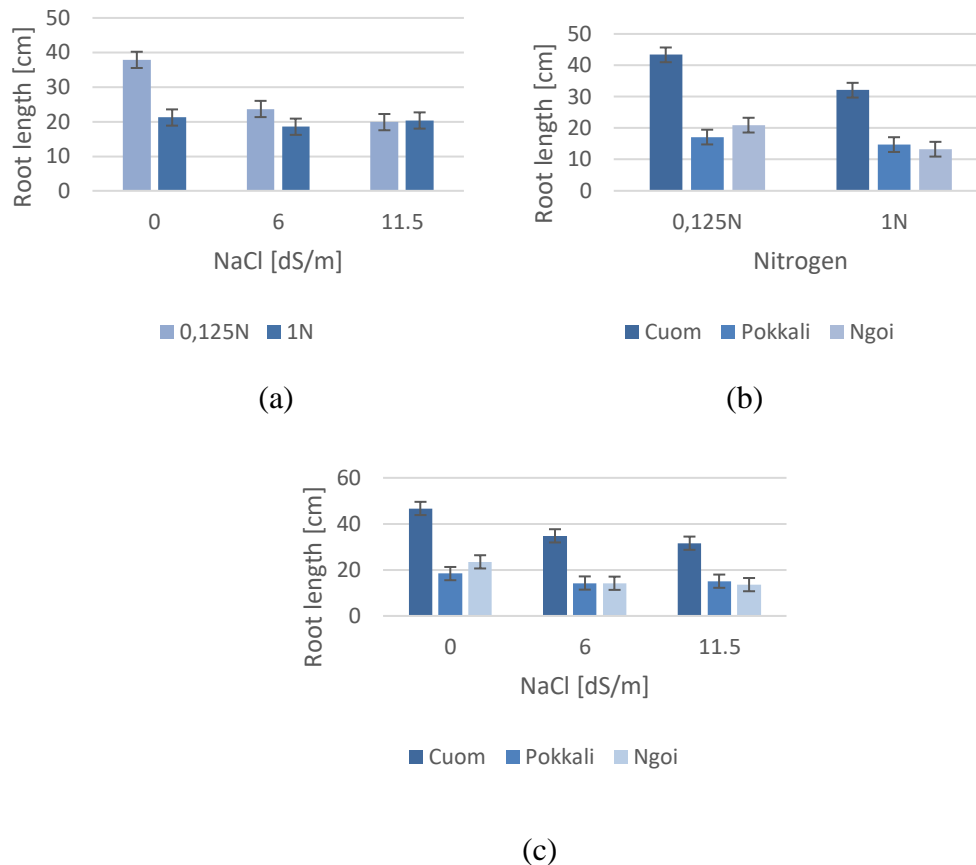


Figure 11: Root length at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the N concentration for the 3 genotypes; (c) in function of the salt concentration for the 3 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

## 1.2.2. Physiological Adjustment

The analysis was focused on the shoots for this part. Most of the physiological functions analyzed were only present in the shoots. The other variables had very low

content levels in the roots, and therefore the methods used to quantify them were not adapted or usually not accurate enough. To avoid a useless repetition, when no precision was made about a root analysis, all the data were obtained on the shoots.

### 1.2.2.1. Effects on Photosynthesis Function

#### 1.2.2.1.1. Assimilation Rate of CO<sub>2</sub>

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the assimilation rate of CO<sub>2</sub> (Pr > F: 0.8942), therefore the restrained model was calculated. The restrained model was not suitable for analysis (R<sup>2</sup>= 0.59).

#### 1.2.2.1.2. Transpiration Rate

The ANOVA (Table 19) of the complete model showed that the triple interaction parameter was not significant on the transpiration rate, therefore the restrained model was calculated. The restrained model was suitable for analysis (R<sup>2</sup>= 0.73). Many parameters were significant on the transpiration, the interactions N-NaCl and genotype-N were not significant.

Table 19: ANOVA models of the transpiration rate at 4 weeks of treatment

<i>Type III ANOVA - Transpiration rate</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.0005	0.2009	0.6174	0.0188	0.0849
Pr > F	<.0001	<.0001	0.0012	0.1139	0.5583	0.0243	

**Genotype influence:** The genotype had a significant influence on the transpiration rate. The cultivars were significantly different from each other, Cuom had the highest rate, followed by Pokkali and Ngoi (Table 20 **Error! Reference source not found.** and Figure 12).

Table 20: LSMEANS of the transpiration rate in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Transpiration rate [mol m<sup>-2</sup> s<sup>-1</sup>]</i>
<b>Cuom</b>	5.15 ± 0.43
<b>Pokkali</b>	3.84 ± 0.46
<b>Ngoi</b>	2.96 ± 0.48

**NaCl influence:** The salt had a significant negative influence over the transpiration rate (Figure 12). The means rate of the medium salt level and strong salt level corresponded to about 53% and 17% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the transpiration rate. The mean rate of the 0.125 standard N concentration corresponded to 80% of the standard nitrogen one.

**Interaction genotype-NaCl:** The interaction had a significant effect on the transpiration rate. The difference between cultivars was accentuated with the increasing salinity (Figure 12). The most noticeable effect was the evolution of Ngoi, from being on average with the control to nearly cease with the strong salinity.

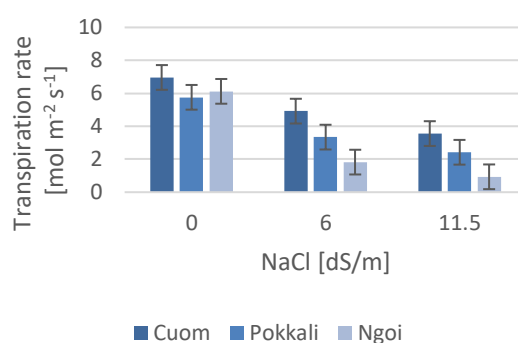


Figure 12: Transpiration rate at 4 weeks of treatment in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.1.3. Stomatal Conductance

The ANOVA (Table 21) of the complete model showed that the triple interaction parameter was not significant on the stomatal conductance, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2= 0.76$ ). Most of the parameters were significant on the transpiration. Only the interaction genotype-N was not significant. The stomatal conductance was highly correlated (86%) with the transpiration rate.

Table 21: ANOVA models of the stomatal conductance at 4 weeks of treatment

<i>Type III ANOVA - Stomatal conductance</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.0002	<.0001	0.6111	0.0012	0.8359
Pr > F	<.0001	<.0001	0.0002	<.0001	0.6058	0.0008	

**Genotype influence:** The genotype had a significant influence on the stomatal conductance. Only Cuom had a significantly higher stomatal conductance than the other cultivars (Table 22 and Figure 13).

Table 22: LSMEANS of the stomatal conductance in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Stomatal conductance [<math>\mu\text{mol m}^{-2} \text{s}^{-1}</math>]</i>
<b>Cuom</b>	0.31 ± 0.03
<b>Pokkali</b>	0.19 ± 0.03
<b>Ngoi</b>	0.16 ± 0.03

**NaCl influence:** The salt had a significant negative influence on the stomatal conductance (Figure 13). The means rate of the medium salt level and strong salt level corresponded to about 54% and 21% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the stomatal conductance (Figure 13). The mean rate of the 0.125 standard N concentration corresponded to 70% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on the stomatal conductance (Figure 13a). The main significant effect was observed for the control, where the stomatal conductance was higher with the standard N concentration, whereas no effect of N concentration was noticeable in the presence of NaCl. The other salt levels were not influenced by the N.

**Interaction genotype-NaCl:** The interaction had a significant effect for the stomatal conductance. The difference between cultivars was accentuated with the increasing salinity and was not significant for the control (Figure 13b).

#### 1.2.2.1.4. Sub-Stomatal CO<sub>2</sub> Concentration

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the sub-stomatal CO<sub>2</sub> concentration (Pr > F: 0.1266), therefore the restrained model was calculated. The restrained model was not suitable for analysis (R<sup>2</sup>= 0.32).

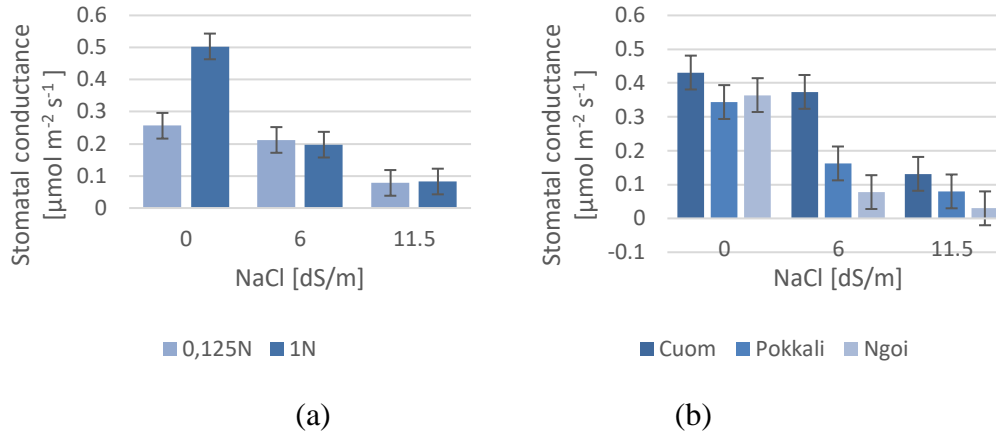


Figure 13: Stomatal conductance at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.2. Total Protein Content in Shoot

The ANOVA (Table 23) of the complete model showed that the triple interaction parameter was not significant on the total protein content in shoot, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2=0.73$ ). All the parameters were significant on the protein content in shoot.

Table 23: ANOVA models of the total protein content in shoot at 4 weeks of treatment

<i>Type III ANOVA - Total protein content in shoot</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	0.0013	<.0001	0.0002	0.0001	0.0277	0.1499
Pr > F	<.0001	0.0020	<.0001	0.0002	0.0002	0.0308	

**Genotype influence:** The genotype had a significant influence on the protein content in shoot. All the cultivars were significantly different from each other with Cuom having the highest protein content, followed by Pokkali and Ngoi (Table 24 and Figure 14).

Table 24: LSMEANS of the total protein content in shoot in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Total protein content in shoot [mg g<sup>-1</sup>MF]</i>
<b>Cuom</b>	2.78 ± 0.18
<b>Pokkali</b>	2.03 ± 0.18
<b>Ngoi</b>	1.44 ± 0.18

**NaCl influence:** The salt had a significant variable influence on the protein content in shoot (Figure 14). The medium salinity had the highest content before the control and finally the strong salt condition (significantly different from the medium salinity and nearly to the control).

**N influence:** The nitrogen had a significant positive effect on the protein content in shoot (Figure 14). The mean rate of the 0.125 standard N concentration corresponded to 63% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on the protein content in shoot (Figure 14a). The effect of the NaCl was decreased by the positive N effect. However, no significant difference by N can be observed on the medium salt condition.

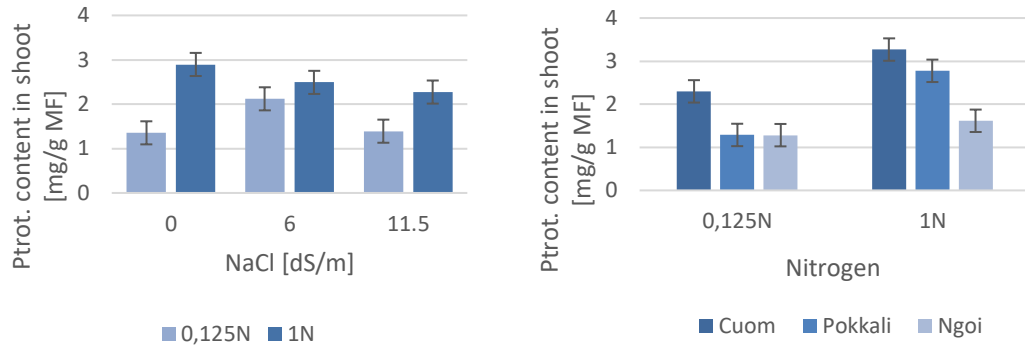
**Interaction genotype-N:** The interaction had a significant effect on the protein content in shoot (Figure 14b). The positive effect of N can be observed, but the effect of the genotype is mainly noticeable for the standard nitrogen concentration (Pokkali and Ngoi were similar under the low N concentration).

**Interaction genotype-NaCl:** The interaction had a significant effect on the protein content in shoot. The trends of the genotype effect can be observed (Cuom has the highest content, followed by Pokkali and Ngoi) and became significant with the increasing salt condition (Figure 14c). Cuom had a higher protein content under salt stress than for the control.

### **1.2.2.1. Total Protein Content in Roots**

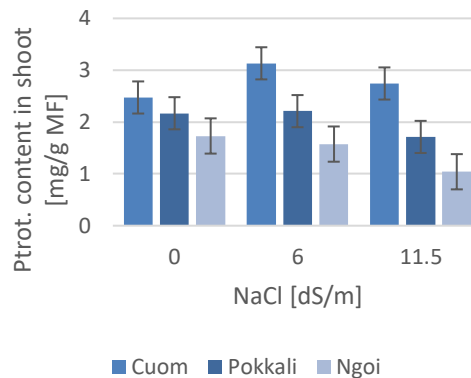
The ANOVA of the complete model showed that the triple interaction parameter was not significant on the protein content in roots ( $P > F: 0.1915$ ), therefore the restrained model was calculated. The restrained model was less suitable for analysis ( $R^2 = 0.60$ ) and the fit diagnostics were not so conclusive.

The protein content extracted from the roots was very low on the calibration curve and therefore the analysis was difficult.



(a)

(b)



(c)

Figure 14: Total protein content in shoot at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the N concentration for the 3 genotypes; (c) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

## 1.2.2.2. Chlorophyll Content

### 1.2.2.2.1. $\alpha$ -Chlorophyll Content

The ANOVA (Table 25) of the complete model showed that the triple interaction parameter was significant on the  $\alpha$ -chlorophyll content. The complete model was suitable for analysis ( $R^2= 0.72$ ). Almost all the parameters were significant on the  $\alpha$ -chlorophyll content. Only the interaction genotype-N was not significant.

**Genotype influence:** The genotype had a significant influence on the  $\alpha$ -chlorophyll content. Only Cuom was significantly superior to the other cultivars (Table 26).

Table 25: ANOVA model of the  $\alpha$ -chlorophyll content at 4 weeks of treatment

<i>Type III ANOVA - <math>\alpha</math>-chlorophyll content</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	<.0001	0.2069	<.0001	0.0073

Table 26: LSMEANS of the  $\alpha$ -chlorophyll content in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - <math>\alpha</math>-chlorophyll content [mg g<sup>-1</sup>MF]</i>
<b>Cuom</b>	0.62 ± 0.03
<b>Pokkali</b>	0.39 ± 0.03
<b>Ngoi</b>	0.38 ± 0.03

**NaCl influence:** The salt had a significant influence on the  $\alpha$ -chlorophyll content. The three salt conditions were significantly different from each other. The means rate of the medium salt level and strong salt level corresponded to about 77% and 38% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the  $\alpha$ -chlorophyll content (Figure 15a). The mean rate of the 0.125 standard N concentration corresponded to 61% of the standard nitrogen one.

**Interaction N-NaCl:** The effect of the interaction was significant on the  $\alpha$ -chlorophyll content (Figure 15a). The positive effect of N was attenuated with the increasing salinity. The low N concentration had similar results for the control and under the medium salinity.

**Interaction genotype-NaCl:** The interaction had a significant effect on the  $\alpha$ -chlorophyll content (Figure 15b). The trends were difficult to characterize, the increasing salinity provoked an inversion of the higher  $\alpha$ -chlorophyll content between Pokkali and Ngoi (lower one at the end), while Cuom had always the highest content.

**Triple interaction:** The triple interaction had a significant effect, but it was difficult to notice the influence.

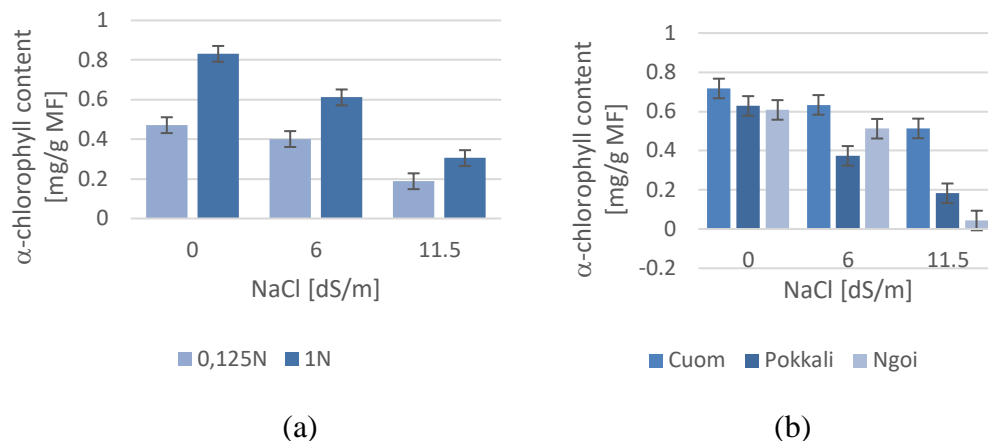


Figure 15:  $\alpha$ -chlorophyll content at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.2.2. $\beta$ -Chlorophyll Content

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the  $\beta$ -chlorophyll content ( $Pr > F: 0.9735$ ), therefore the restrained model was calculated. The restrained model was not suitable for analysis ( $R^2=0.003$ ).

The contents were very low and therefore difficult to quantify correctly.

### 1.2.2.2.3. Carotenoid Content

The ANOVA of the complete model showed that the triple interaction parameter was significant on the carotenoid content ( $Pr > F: 0.0106$ ). The complete model was less suitable for analysis ( $R^2=0.68$ ) than the  $\alpha$ -chlorophyll one. As the carotenoid content were highly correlated to the  $\alpha$ -chlorophyll content (97%), the trends observed were similar and therefore the analysis were not repeated in this section.

### 1.2.2.3. Nitrogen Content

The ANOVA (Table 27) of the complete model showed that the triple interaction parameter was significant on the nitrogen content. The complete model was suitable for analysis ( $R^2=0.93$ ). All the parameters were significant on the nitrogen content.

Table 27: ANOVA model of the nitrogen content at 4 weeks of treatment

<i>Type III ANOVA - nitrogen content</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	0.0351	<.0001	<.0001	0.0270	<.0001	0.0243

**Genotype influence:** The genotype had a significant influence on the nitrogen content. Only Cuom was significantly higher than the other cultivars (Table 28 and Figure 16b).

Table 28: LSMEANS of the nitrogen content in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - nitrogen content [%]</i>
<b>Cuom</b>	3.24 ± 0.08
<b>Pokkali</b>	2.60 ± 0.08
<b>Ngoi</b>	2.68 ± 0.08

**NaCl influence:** The salt had a significant positive influence on the nitrogen content. The salinity levels results were not significantly different from each other, but the control had a lower content than the others (Figure 16). The means rate of the medium salt level and strong salt level corresponded to 105% and 104% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the nitrogen content (Figure 16a-b). The mean rate of the 0.125 standard N concentration corresponded to 68% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on the nitrogen content (Figure 16a). Rising the NaCl concentration resulted in a decrease and a rise of the nitrogen content for the standard N and the low N concentrations, respectively.

**Interaction genotype-N:** The interaction had a significant effect on the nitrogen content (Figure 16b). Low effects were observed on the genotypes with only Cuom being significantly higher than the others, but the positive N influence remained.

**Interaction genotype-NaCl:** The interaction had a significant effect on the nitrogen content (Figure 16c). The trends were difficult to characterize, the only significantly different cultivar from the others was Cuom being higher under the medium and strong salinity.

### 1.2.2.1. Carbon Nitrogen Ratio

The ANOVA (**Error! Reference source not found.**) of the complete model showed that the triple interaction parameter was not significant on the carbon nitrogen (C/N) ratio, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2= 0.92$ ). All the parameters were significant on the carbon/nitrogen ratio. The ratio was negatively correlated to the N contents with 87%.

Table 29: ANOVA models of the carbon/nitrogen ratio at 4 weeks of treatment

<i>Type III ANOVA - carbon/nitrogen ratio</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	<.0001	0.0139	0.0113	0.1286
Pr > F	<.0001	<.0001	<.0001	<.0001	0.0101	0.0155	

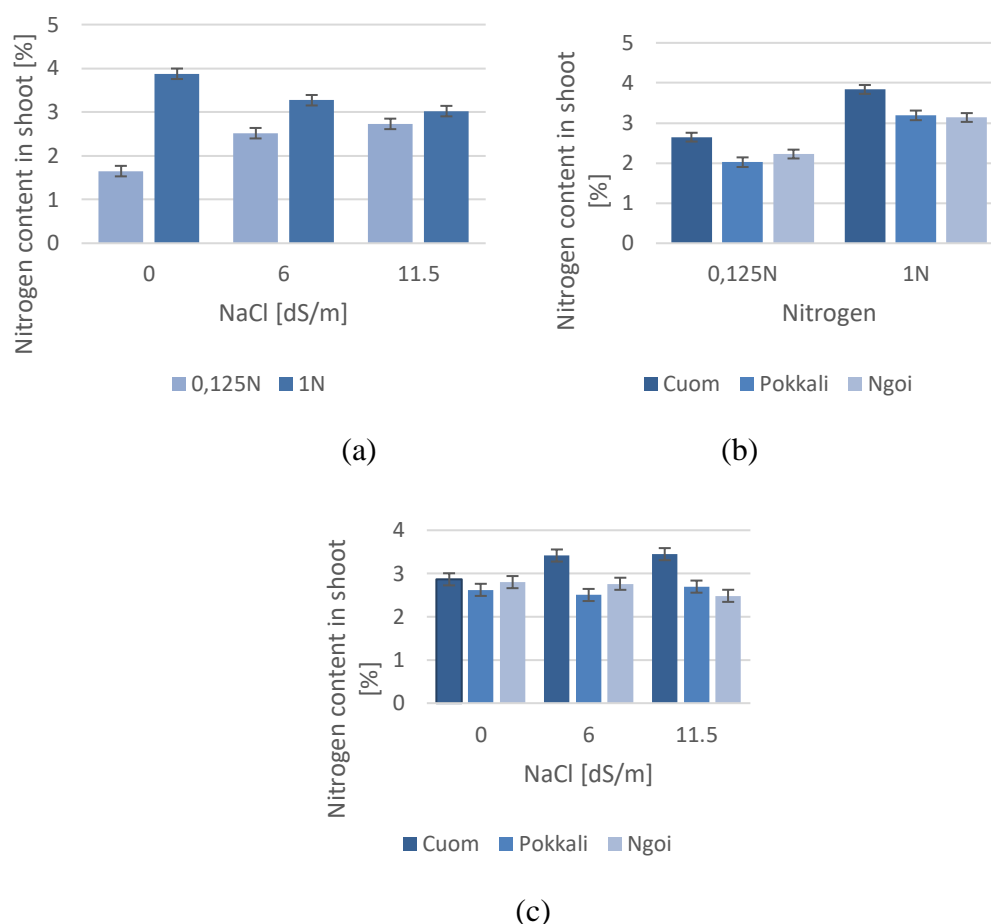


Figure 16: Nitrogen content at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the salt concentration for the 3 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

**Genotype influence:** The genotype had a significant influence on the C/N ratio. Only Pokkali had a significantly higher C/N ratio than the other cultivars (Table 30 and Figure 17b).

Table 30: LSMEANS of the carbon/nitrogen ratio in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - C/N ratio</i>
<b>Cuom</b>	14.07 ± 0.53
<b>Pokkali</b>	16.32 ± 0.55
<b>Ngoi</b>	14.94 ± 0.54

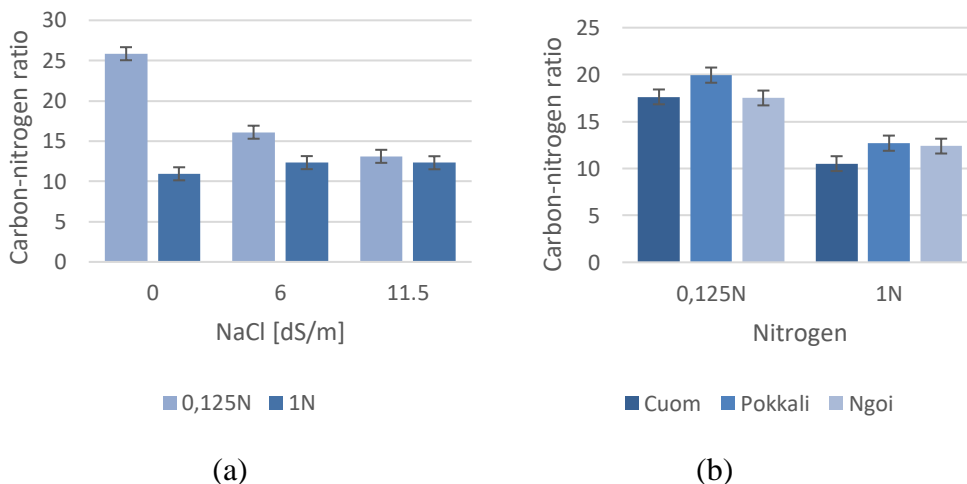
**NaCl influence:** The salt had a significant negative influence on the C/N ratio (Figure 17). The means rate of the medium salt level and strong salt level corresponded to about 77% and 69% of the control, respectively.

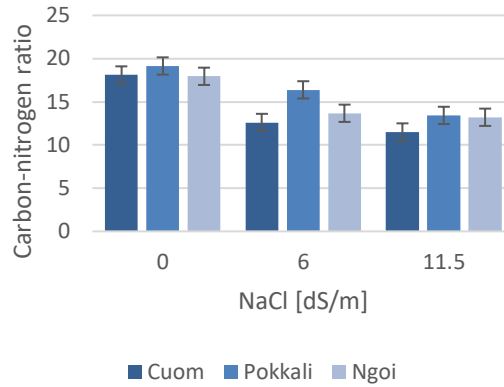
**N influence:** The nitrogen had a significant negative effect on the C/N ratio (Figure 17a-b). The mean rate of the 0.125 standard N concentration corresponded to 155% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on the C/N ratio (Figure 17a). Rising the NaCl concentration resulted in a decrease and a rise of the C/N ratio for the low N and the standard N concentrations, respectively.

**Interaction genotype-N:** The interaction had a significant effect on the C/N ratio (Figure 17b). However, the effects were low and difficult to identify

**Interaction genotype-NaCl:** The interaction had a significant effect on the C/N ratio (Figure 17c). Again, the trends were difficult to characterize, only Pokkali showed a higher C/N ratio than the other cultivars under the medium salinity.





(c)

Figure 17: Carbon Nitrogen ratio at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the N concentration for the 3 genotypes; (c) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.2. Nitrogen Use Efficiency

The ANOVA (Table 31) of the complete model showed that the triple interaction parameter was significant on the NUE, excepted for the physiological NUE.

Table 31: ANOVA models of the nitrogen use efficiency at 4 weeks of treatment

<i>Type III ANOVA - Absorption NUE of shoot</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	0.0201	<.0001	0.1135	<.0001	0.0009	0.0063
<i>Type III ANOVA - Physiological NUE of shoot</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	<.0001	0.0037	<.0001	0.1023
Pr > F	<.0001	<.0001	<.0001	<.0001	0.0066	<.0001	
<i>Type III ANOVA - Agronomical NUE</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	0.0023	0.0299

#### 1.2.2.2.1. Absorption Nitrogen Use Efficiency

The complete model was suitable for analysis ( $R^2 = 0.93$ ). Almost all the parameters were significant on the shoot aNUE. Only the interaction N-NaCl was not significant.

**Genotype influence:** The genotype had a significant influence on the aNUE. The only difference was striking: Cuom had a significantly very higher aNUE than the other cultivars (Table 32 and Figure 18b).

Table 32: LSMEANS of the absorption NUE in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Absorption NUE [%]</i>
<b>Cuom</b>	22.41 ± 1.97
<b>Pokkali</b>	5.53 ± 1.97
<b>Ngoi</b>	6.84 ± 2.07

**NaCl influence:** The salt had a significant negative influence on aNUE. Only the strongest salinity was significantly different from the other salinity levels. The means rate of the medium salt level and strong salt level corresponded respectively to 91% and 70% of the control.

**N influence:** The nitrogen had a significant negative effect on the aNUE (Figure 18a). The mean rate of the standard N concentration corresponded to 28% of the 0.125 standard nitrogen one.

**Interaction genotype-N:** The interaction had a significant effect on the aNUE (Figure 18a). Low effects were observed between Pokkali and Ngoi, but the N negative influence was very important on Cuom.

**Interaction genotype-NaCl:** The interaction had a significant effect on the aNUE (Figure 18b). The trends were difficult to characterize, and the effects were variable. Cuom had its highest value at the medium salinity but remained very efficient for each salinity level. Pokkali and Ngoi were not significantly different from each other and kept a low efficiency on the different salinity levels; only Ngoi decreased significantly from the control to the other salinity levels).

**Triple interaction:** The triple interaction had a significant effect, but it was difficult to notice the specific influence.

Overall, Cuom behaved differently from the other cultivars. Its aNUE was by far higher compared to the other cultivars, whatever the salinity concentration; The major difference was reached under the reduced N applied level and rose up to 36%.

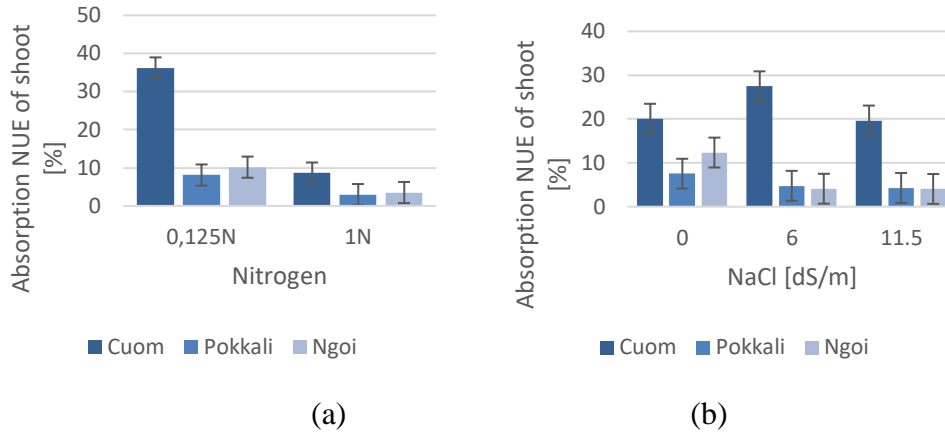


Figure 18: Absorption NUE at 4 weeks of treatment: (a) in function of the N concentration for the 3 genotypes; (b) in function of the salt concentration for the 3 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

#### 1.2.2.2.2. Physiological Nitrogen Use Efficiency

The complete model was suitable for analysis ( $R^2 = 0.92$ ) All the parameters were significant on the pNUE.

**Genotype influence:** The genotype had a significant influence on the pNUE. All the cultivars were significantly different from each other (Table 33 and Figure 19b). Pokkali had the highest value before Ngoi and finally Cuom.

Table 33: LSMEANS of the physiological NUE in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Physiological NUE [kg kg<sup>-1</sup>]</i>
<b>Cuom</b>	34.28 ± 1.39
<b>Pokkali</b>	42.08 ± 1.39
<b>Ngoi</b>	39.08 ± 1.45

**NaCl influence:** The salt had a significant negative influence on pNUE. Only the control was significantly different from the other cultivars (Figure 19). The means rate of the medium salt level and strong salt level corresponded respectively to about 84% and 82% of the control.

**N influence:** The nitrogen had a significant negative effect on pNUE (Figure 19a). The mean rate of the standard N concentration corresponded to 65% of the 0.125 standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on pNUE (Figure 16a). Raising the NaCl concentration resulted in a decrease and a rise of the pNUE for the low N and standard N concentrations, respectively.

**Interaction genotype-N:** The interaction had a significant effect on the pNUE. Trends were difficult to characterize, the N influence remained.

**Interaction genotype-NaCl:** The interaction had a significant effect on the pNUE (Figure 19b). The trends were difficult to characterize, Ngoi remained constant no matter salinity was present or not. The two salt-tolerant cultivars had lower efficiency when a salinity was applied and Cuom was significantly lower than the others under medium and strong salinity.

**Triple interaction:** The triple interaction had a significant effect, but it was difficult to notice the influence.

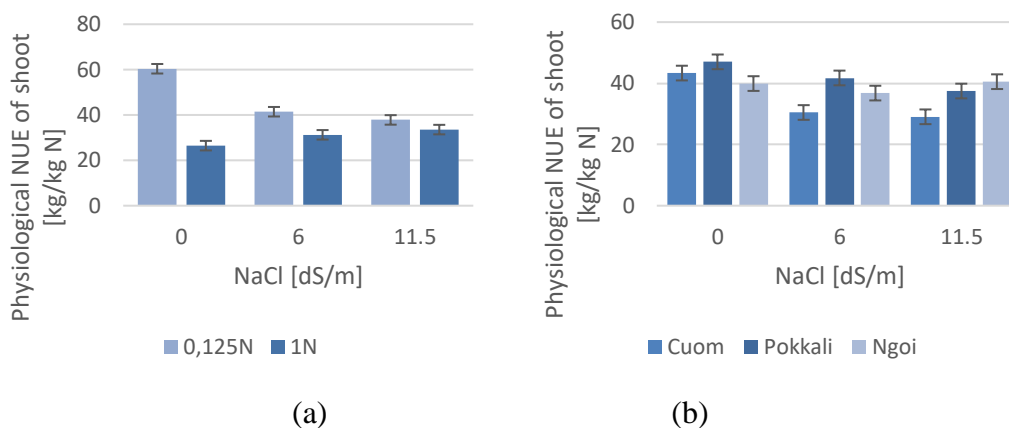


Figure 19: Physiological NUE at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the salt concentration for the 3 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.2.3. Agronomical Nitrogen Use Efficiency

The restrained model was suitable for analysis ( $R^2 = 0.93$ ). All the parameters were significant on the agNUE.

**Genotype influence:** The genotype had a significant influence on the agNUE. Only Cuom was significantly higher than the other cultivars (Table 28 and Figure 20).

Table 34: LSMEANS of the agronomical NUE in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Agronomical NUE [kg kg<sup>-1</sup>]</i>
<b>Cuom</b>	9.97 ± 0.69
<b>Pokkali</b>	2.95 ± 0.69
<b>Ngoi</b>	3.42 ± 0.69

**NaCl influence:** The salt had a significant negative influence on agNUE. The salinity levels results were significantly different from each other (Figure 20). The means rate of the medium salt level and strong salt level corresponded to about 60% and 43% of the control, respectively.

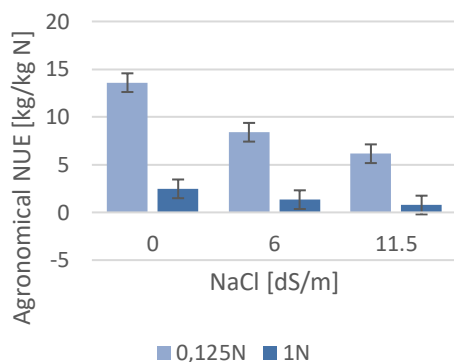
**N influence:** The nitrogen had a significant negative effect on agNUE (Figure 20). The mean rate of the standard N concentration corresponded to 16% of the 0.125 nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on agNUE (Figure 20a). The negative effects of increasing NaCl concentration were more marked under low N concentration.

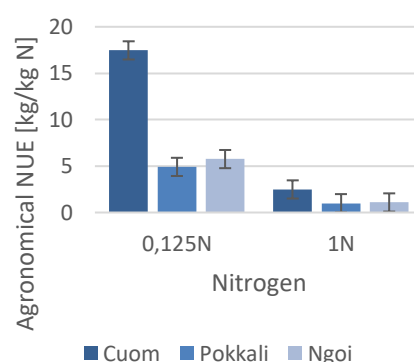
**Interaction genotype-N:** The interaction had a significant effect on agNUE (Figure 20b). Cuom had a much higher AgNUE than the other genotypes under 0.125N.

**Interaction genotype-NaCl:** The interaction had a significant effect on agNUE (Figure 20c). Ngoi was slightly more efficient without salt than Pokkali but was not significantly different from it under moderate or high salt treatment.

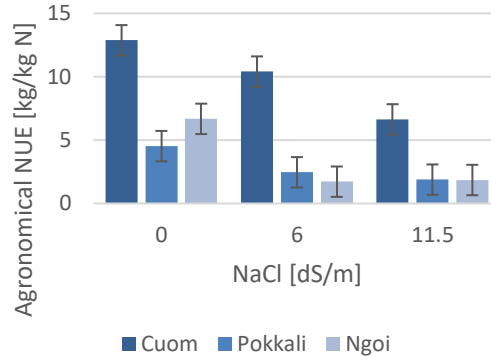
**Triple interaction:** The triple interaction had a significant effect, but it was difficult to notice the influence.



(a)



(b)



(c)

Figure 20: Agronomical NUE at 4 weeks of treatment: (a) in function of the salt concentration for the 2 N concentrations; (b) in function of the N concentration for the 3 genotypes; (c) in function of the salt concentration for the 3 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.3. Na Content

The ANOVA (Table 35) of the complete model showed that the triple interaction parameter was significant on the sodium content. The complete model was suitable for analysis ( $R^2 = 0.87$ ). Almost all the parameters were significant on the Na content. Only the interaction N-NaCl was not significant.

Table 35: ANOVA model of the sodium content at 4 weeks of treatment

<i>Type III ANOVA - Sodium content</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.0075	<.1351	0.0035	<.0001	0.0315

**Genotype influence:** The genotype had a strong and significant influence on the Na content. All the cultivars were significantly different from each other (Table 36 and Figure 21). The salt sensitive cultivar Ngoi had the highest sodium content; Cuom had the lowest one.

Table 36: LSMEANS of the sodium content in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Sodium contents [g/kg dry matter]</i>
<b>Cuom</b>	12.5 ± 4.3
<b>Pokkali</b>	25.2 ± 4.3
<b>Ngoi</b>	40.1 ± 4.3

**NaCl influence:** The salt had a significant positive influence on the Na content (Figure 21b), as expected. The means rate of the control and medium salt level corresponded to 2% and 44% of the strong salt level, respectively.

**N influence:** The nitrogen had a significant negative effect on the Na content (Figure 21a). The mean rate of the 0.125 standard N concentration corresponded to 130% of the standard nitrogen one.

**Interaction genotype-N:** The interaction had a significant effect on the Na content (Figure 21a). The differences between cultivars were more marked under low N concentration.

**Interaction genotype-NaCl:** The interaction had a significant effect on the Na content (Figure 21b). The trends followed the effects of the genotype and salinity.

**Triple interaction:** The triple interaction had a significant effect, but it was difficult to notice the influence.

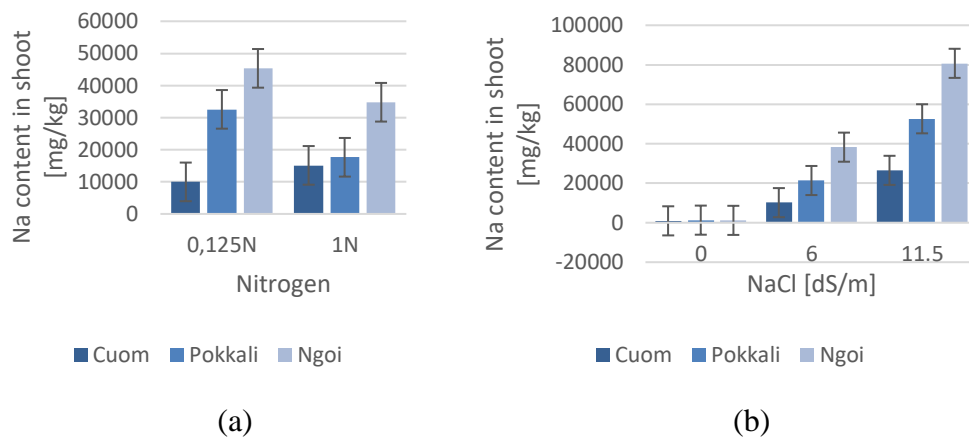


Figure 21: Sodium content at 4 weeks of treatment: (a) in function of the nitrogen concentration for the 3 genotypes; (b) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.4. K Content

The ANOVA (Table 37) of the complete model showed that the triple interaction parameter was not significant on the potassium content, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2 = 0.79$ ). Many

parameters were significant on the potassium content. The interactions genotype-N and genotype-NaCl were not significant.

Table 37: ANOVA models of the potassium content at 4 weeks of treatment

<i>Type III ANOVA - potassium content</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.0008	0.0211	0.3843	0.4905	0.6757
Pr > F	<.0001	<.0001	0.0007	0.0192	0.3760	0.4787	

**Genotype influence:** The genotype had a significant influence on the K content. All the cultivars were significantly different from each other (Table 38). Contrary to what was observed for Na content, Cuom had the lowest K rate, followed by Pokkali and finally Ngoi

Table 38: LSMEANS of the potassium content in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - potassium content [g/kg]</i>
<b>Cuom</b>	37 ± 2
<b>Pokkali</b>	28 ± 2
<b>Ngoi</b>	23 ± 2

**NaCl influence:** The salt had a significant negative influence on the K content. The results were significantly different from each other (Figure 22). The means rate of the medium salt level and strong salt level corresponded to 65% and 55% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the K content (Figure 22). The mean rate of the 0.125 standard N concentration corresponded to 87% of the standard nitrogen one.

**Interaction N-NaCl:** The interaction had a significant effect on the K content (Figure 22). The difference of K absorption between the N concentrations was only observed for the salt control.

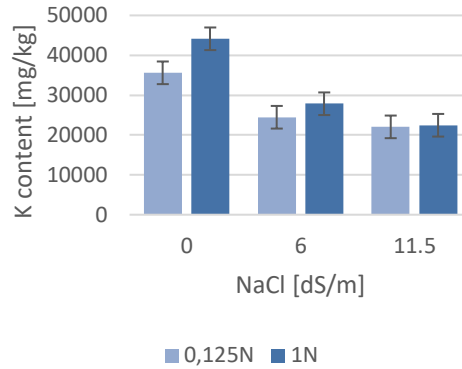


Figure 22: Potassium content at 4 weeks of treatment in function of the salt concentration for the 2 N concentrations. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.5. Sodium Potassium Ratio

The ANOVA (Table 39) of the complete model showed that the triple interaction parameter was not significant on the sodium potassium ratio, therefore the restrained model was calculated. The restrained model was suitable for analysis ( $R^2= 0.84$ ). Almost all the parameters were significant on the Na/K ratio. Only the interaction N-NaCl was not significant. The ratio was negatively correlated to the Na contents with 98%.

Table 39: ANOVA models of the Na/K ratio at 4 weeks of treatment

<i>Type III ANOVA - Na/K ratio</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	0.0031	0.0678	0.0115	<.0001	0.0750
Pr > F	<.0001	<.0001	0.0040	0.0790	0.0147	<.0001	

**Genotype influence:** The genotype had a significant influence on the Na/K ratio. All the cultivars were significantly different from each other (Table 40 **Error! Reference source not found.** and Figure 23). The salt sensitive cultivar Ngoi had the highest Na/K ratio; Cuom had the lowest one.

Table 40: LSMEANS of the Na/K ratio in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Na/K ratio</i>
<b>Cuom</b>	0.43 ± 0.26
<b>Pokkali</b>	1.21 ± 0.26
<b>Ngoi</b>	2.25 ± 0.26

**NaCl influence:** The salt had a significant and positive influence on the Na/K ratio. The results were significantly different from each other (Figure 23b). The means rate of the control and medium salt level corresponded to 1% and 37% of the strong salt condition, respectively.

**N influence:** The nitrogen had a significant negative effect on the Na/K ratio (Figure 23a). The mean rate of the standard N concentration corresponded to 70% of the 0.125 nitrogen one.

**Interaction genotype-N:** The interaction had a significant effect on the Na/K ratio (Figure 23a). The significant effects were observed on the genotype under the low N concentration. Under the standard N, only Ngoi was significantly higher than the other cultivars.

**Interaction genotype-NaCl:** The interaction had a significant effect on the Na/K ratio (Figure 23b). The significant effects were observed on the genotype mainly under the strong salinity.

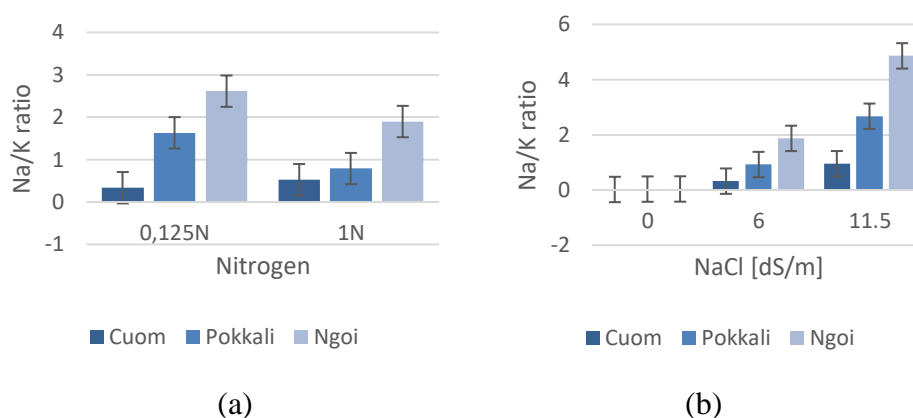


Figure 23: Na/K ratio at 4 weeks of treatment: (a) in function of the nitrogen concentration for the 3 genotypes; (b) in function of the salt concentration for the 3 genotypes. The restrained GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar Pokkali: Reference trad. - tolerant. 0,125N = 0.356 mM N; 1N = 2.86 mM N.

### 1.2.2.6. Cl Content

Preliminary tests to analyze the chloride content were conducted on a few samples of the strong salinity and the no salt condition (protocol in annex 1). Unfortunately, the result reliability was not conclusive and therefore the analysis was not carried out.

As the mineralization had to be done with 0.5% of nitric acid, the samples had to be diluted 50 times before analysis to avoid the saturation of the ion chromatography column. The results obtained were very low and just at the detection limit for the analyzer. Moreover, the nitrate peak area was very high and could cover peaks of the other elements analyzed. The results of the preliminary tests are in annex 3.

## 2 Experiment in Vietnam

In this experiment, two additional intermediary nitrogen concentrations were applied to a total of four cultivars (two salt-tolerant: Cuom, FL478 and two salt-sensitive: Ngoi, IR28) in comparison with the experiments in hydroponics (two salt-tolerant: Cuom, Pokkali and one salt sensitive: Ngoi). The analysis was focused on the active tillering stage to have the best correlation possible with the experiment in hydroponics. Complementary results on the heading stage are presented in annex 3.

### 2.1 Morphological adjustment

#### 2.1.1. Shoot Dry Weight

The ANOVA (Table 41) of the complete model showed that the triple interaction parameter was significant on the shoot dry weight. The complete model was suitable for analysis ( $R^2= 0.83$ ). All the parameters were significant on the shoot dry weight. The total dry weight was highly correlated to this variable (95%).

Table 41: ANOVA model of the shoot dry weight at active tillering

<i>Type III ANOVA - Shoot dry weight</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	<.0001	0.0030	0.0006	0.0012

**Genotype influence:** The genotype has a significant effect on the shoot dry weight (Table 42 and Figure 24b). FL478 dry weight was higher than the others. However, the other salt-tolerant cultivar, Cuom, presented in general a lower result than Ngoi (salt-sensitive).

**NaCl influence:** The salt had a significant negative influence over the shoot dry weight (Figure 24). The means dry weights of the medium salt level and strong salt level corresponded to 63% and 24% of the control, respectively.

Table 42: LSMEANS of the shoot dry weight at active tillering

	<i>LSMEANS - Shoot dry weight [g]</i>
<b>Cuom</b>	1.70 ± 0.18
<b>FL478</b>	2.12 ± 0.17
<b>IR28</b>	1.47 ± 0.18
<b>Ngoi</b>	1.78 ± 0.21

**N influence:** The nitrogen had a significant positive effect on the shoot dry weight (Figure 24a). The means dry weight of the 0.125N, 0.25N and 0.5N concentrations corresponded to 25%, 38% and 71% of the standard nitrogen one, respectively.

**Interaction N-NaCl:** The interaction had a significant effect on the shoot dry weight (Figure 24a). The trends observed for the separated parameters (the positive effect of N and the negative of NaCl) were present but they were slightly attenuated for the medium and the strong NaCl level.

**Interaction genotype-N:** The interaction had a significant effect on the dry shoot weight. The effects of the interaction were difficult to characterize.

**Interaction genotype-NaCl:** The interaction had a significant effect on the dry shoot weight (Figure 24b). Under no or moderate salt concentration, all cultivars behaved similarly, but FL478 was significantly superior to the other cultivars under the strong salinity.

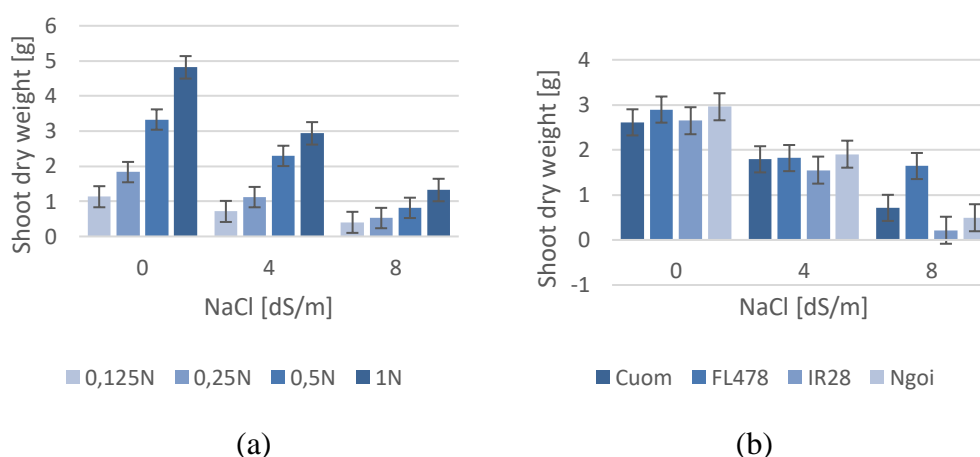


Figure 24: Shoot weight at active tillering: (a) in function of the salt concentration for the 4 N concentrations; (b) in function of the salt concentration for the 4 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar FL478: improved - tolerant; cultivar IR28: improved - sensitive. 0,125N = 0.125g N; 0,25 = 0,25g N; 0,5N = 0,5g N; 1N = 1g N.

### 2.1.2. Root Dry Weight

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the root weight (Pr > F: 0.0523), therefore the restrained model was calculated. The restrained model was not suitable for analysis ( $R^2= 0.50$ ).

### 2.1.3. Shoot/Root Dry Weight Ratio

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the root weight (Pr > F: 0.2266), therefore the restrained model was calculated. The restrained model was not suitable for analysis ( $R^2= 0.20$ ).

### 2.1.4. Shoot Length

The ANOVA (Table 43) of the complete model showed that the triple interaction parameter was significant on the shoot length. The complete model was suitable for analysis ( $R^2= 0.80$ ). Most of the parameters were significant on the shoot length. Only the interactions N-NaCl and genotype-N were not significant.

Table 43: ANOVA model of the shoot length at active tillering (5 weeks of treatment)

<i>Type III ANOVA - Shoot length</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	<.0001	<.0001	0.1051	0.4075	<.0001	0.0140

**Genotype influence:** The genotype had a significant influence on the shoot length (Table 44 and Figure 25). The cultivars were significantly different from each other excepted for the two salt-tolerant genotypes. Ngoi had the highest length, while the other salt-sensitive cultivar (IR28) had the lowest.

Table 44: LSMEANS of the shoot length in function of the 3 genotypes at active tillering (5 weeks of treatment)

	<i>LSMEANS - Shoot length [cm]</i>
<b>Cuom</b>	56 ± 2
<b>FL478</b>	53 ± 2
<b>IR28</b>	45 ± 2
<b>Ngoi</b>	62 ± 2

**NaCl influence:** The salt had a significant negative influence on the shoot length (Figure 25). The means dry weights, significantly different, of the medium salt level and strong salt level corresponded to about 93% and 71% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the shoot length. The means shoot length of the 0.125N, 0.25N and 0.5N concentrations corresponded to 81%, 88% and 95% of the standard nitrogen one, respectively. Therefore, only the 0.5N and the standard N were not significantly different from each other.

**Interaction genotype-NaCl:** The interaction had a significant effect on the shoot length (Figure 25). The negative impact of the salt concentration can be observed for all the cultivars excepted for FL478 which remained similar whatever the salt concentration. The influence of the genotype was significant under strong salinity. However, Ngoi maintained a high shoot length under high salinity.

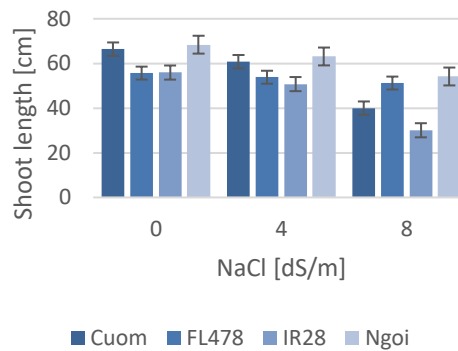


Figure 25: Shoot length at active tillering (5 weeks of treatment) in function of the salt concentration for the 4 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar FL478: improved - tolerant; cultivar IR28: improved - sensitive.

### 2.1.5. Number of tillers

The ANOVA (Table 45) of the complete model showed that the triple interaction parameter was significant on the number of tillers. The complete model was suitable for analysis ( $R^2= 0.83$ ). Almost all the parameters were significant on the number of tillers. Only the interaction genotype-N was not significant.

Table 45: ANOVA model of the number of tillers at active tillering (5 weeks of treatment)

<i>Type III ANOVA - Shoot length</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl

Pr > F	<.0001	<.0001	<.0001	<.0001	0.1378	<.0001	<.0001
--------	--------	--------	--------	--------	--------	--------	--------

**Genotype influence:** The genotype had a significant influence on the number of tillers (Table 46 and Figure 26b). Only FL478 was significantly different and higher from the other cultivars.

Table 46: LSMEANS of the number of tillers in function of the 3 genotypes at active tillering (5 weeks of treatment)

	<i>LSMEANS - Number of tillers</i>
<b>Cuom</b>	4.28 ± 0.41
<b>FL478</b>	5.15 ± 0.35
<b>IR28</b>	3.99 ± 0.37
<b>Ngoi</b>	4.32 ± 0.41

**NaCl influence:** The salt had a significant negative influence on the number of tillers (Figure 26). The means number of tillers, significantly different, of the medium salt level and strong salt level corresponded to 70% and 37% of the control, respectively.

**N influence:** The nitrogen had a significant positive effect on the number of tillers (Figure 26a). The means number of the 0.125N, 0.25N and 0.5N concentrations corresponded to 36%, 53% and 80% of the standard nitrogen one, respectively.

**Interaction N-NaCl:** The interaction had a significant effect on the number of tillers (Figure 26a). The salt negative impact and the positive effect of N can be observed. However, an attenuation of the impact for the second parameter can be observed with the increasing salinity.

**Interaction genotype-NaCl:** The interaction had a significant effect on the number of tillers (Figure 26b). The negative impact of the salt concentration can be observed for all the cultivars. The influence of the genotype was significant under strong salinity with FL478 being significantly less affected than the others (Ngoi as well in a smaller proportion).

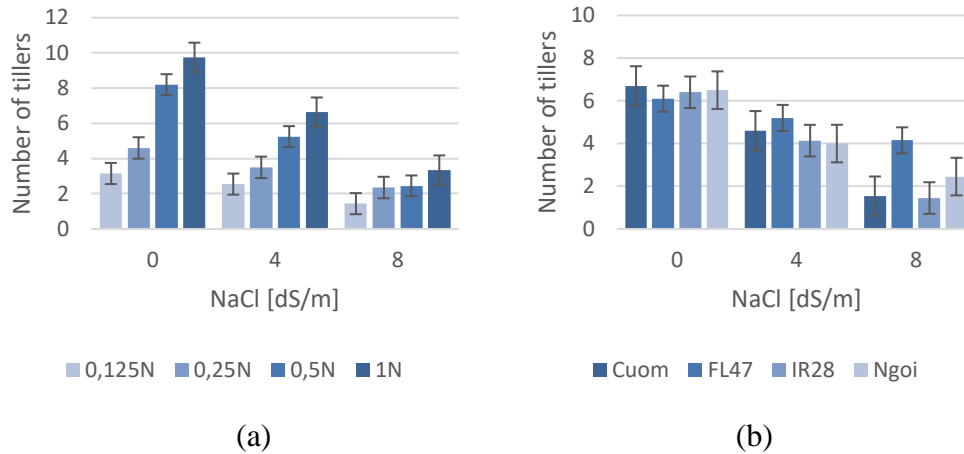


Figure 26: Number of tillers at active tillering (5 weeks of treatment): (a) in function of the salt concentration for the 4 N concentrations; (b) in function of the salt concentration for the 4 genotypes. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar FL478: improved - tolerant; cultivar IR28: improved - sensitive. 0,125N = 0.125g N; 0,25 = 0,25g N; 0,5N = 0,5g N; 1N = 1g N.

## 2.2 Physiological Adjustment

### 2.2.1. Effects on the Photosynthesis Function

Difficulties were encountered to realize the measurements with the gas analyzer and fluorescence system (LICOR 6400). Good climatic conditions (high light intensity) were required to conduct the sampling. Therefore, the measurements were delayed to three weeks after the main active tillering sampling. Unfortunately, too many plants were dead under the strong salinity or not suitable for the measurement, so data were missing for the analysis. In order to conduct the statistical analysis, the strong salt conditions data were put aside.

#### 2.2.1.1. Assimilation Rate of CO<sub>2</sub>

The ANOVA (Table 47) of the complete model showed that the triple interaction parameter was not significant on the assimilation rate of CO<sub>2</sub>, therefore the restrained model was calculated. The restrained model was considered suitable for analysis ( $R^2=0.67$ ) but the results have to be taken cautiously. Many parameters were significant on the assimilation rate of CO<sub>2</sub>, the interactions genotype-N and genotype-NaCl were not significant.

Table 47: ANOVA models of the assimilation rate of CO<sub>2</sub> at active tillering

<i>Type III ANOVA - Assimilation rate of CO<sub>2</sub></i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	<.0001	0.0424	0.0336	0.0245	0.0821	0.2433	0.7917
Pr > F	<.0001	0.0353	0.0250	0.0177	0.0589	0.1919	

**Genotype influence:** The genotype had a significant influence on the assimilation rate. The cultivars were significantly different from each other, excepted for Cuom and Ngoi which had the lowest rate (Table 48). IR28 had the highest assimilation rate, followed by FL478.

Table 48: LSMEANS of the assimilation rate in function of the 3 genotypes at 4 weeks of treatment

	<i>LSMEANS - Assimilation rate [<math>\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}</math>]</i>
<b>Cuom</b>	22.52 ± 1.49
<b>FL478</b>	25.63 ± 1.53
<b>IR28</b>	28.51 ± 1.53
<b>Ngoi</b>	20.99 ± 1.53

**NaCl influence:** The salt had a significant negative influence on the assimilation rate (Figure 27). The mean rate of the medium salt level corresponded to 93% of the control.

**N influence:** The nitrogen had a significant positive effect on the assimilation rate (Figure 27). The mean rate of the 0.125N, 0.25N and 0.5N concentrations corresponded to 88%, 94% and 96% of the standard nitrogen one, respectively.

**Interaction N-NaCl:** The interaction had a significant effect on the assimilation rate (Figure 27). The assimilation rate was independent of N concentration in absence of salt, whereas it rose with N concentration under salt treatment.

### 2.2.1.2. Transpiration Rate

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the root weight (Pr > F: 0.7747), therefore the restrained model was calculated. Unfortunately, it was not suitable for analysis ( $R^2 = 0.50$ ).

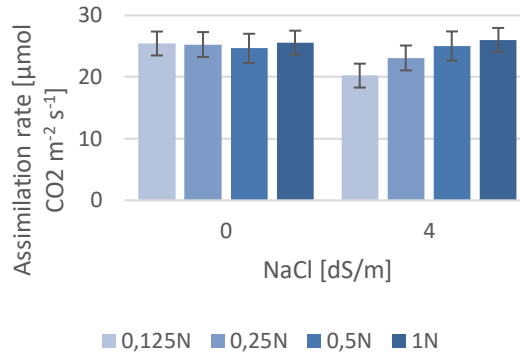


Figure 27: Assimilation rate of CO<sub>2</sub> at active tillering in function of the salt concentration for the 4 N concentrations. The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar FL478: improved - tolerant; cultivar IR28: improved - sensitive. 0,125N = 0.125g N; 0,25 = 0,25g N; 0,5N = 0,5g N; 1N = 1g N.

### 2.2.1.3. Stomatal Conductance

The ANOVA of the complete model showed that the triple interaction parameter was not significant on the root weight (Pr > F: 0.9875), therefore the restrained model was calculated. Unfortunately, it was not suitable for analysis (R<sup>2</sup>= 0.52).

### 2.2.2. Agronomical Nitrogen Use Efficiency

The ANOVA (Table 49) of the complete model showed that the triple interaction parameter was not significant on the agronomical NUE, therefore the restrained model was calculated. The restrained model was considered suitable for analysis (R<sup>2</sup>= 0.65), but the results have to be taken cautiously. Almost all the parameters were significant on the agNUE. Only the interaction genotype-N was not significant.

Table 49: ANOVA models of the agNUE at active tillering

<i>Type III ANOVA - Agronomical NUE</i>							
Parameters	Gen	NaCl	N	N*NaCl	Gen*N	Gen*NaCl	Gen*N*NaCl
Pr > F	0.0004	<.0001	<.0001	0.0071	0.6231	0.0142	0.4663
Pr > F	0.0002	<.0001	<.0001	0.0052	0.3793	0.0112	

**Genotype influence:** The genotype had a significant influence on the agNUE. The two salt-tolerant cultivars (FL478 and Cuom) had slightly higher values but only FL478 was significantly different from the two salt-sensitive genotypes (Table 50 and Figure 28b).

Table 50: LSMEANS of the agronomical NUE in function of the 3 genotypes at active tillering

	<i>LSMEANS - Agronomical NUE [kg kg<sup>-1</sup>]</i>
<b>Cuom</b>	11.15 ± 1.30
<b>FL478</b>	12.97 ± 1.19
<b>IR28</b>	9.09 ± 1.27
<b>Ngoi</b>	10.42 ± 1.43

**NaCl influence:** The salt had a significant negative influence on the agNUE (Figure 28). The salinity levels results were significantly different from each other. The means rate of the medium salt level and strong salt level corresponded to 64% and 27% of the control, respectively.

**N influence:** The nitrogen had a significant negative effect on the agNUE (Figure 28a). Only 0.25N and 0.5N were not significantly different from each other. The mean rate of the standard N, 0.5N, 0.25N concentration corresponded to 40%, 66% and 75% of the 0.125 nitrogen one, respectively.

**Interaction N-NaCl:** The interaction had a significant effect on agNUE (Figure 28a). The decline in agNUE with N applied concentration was the strongest under no salt and the lowest under high salinity.

**Interaction genotype-NaCl:** The interaction had a significant effect on agNUE (Figure 28b). FL478 was higher and significantly different from the other genotypes under strong salinity, while no difference between cultivars was observed under no salt treatment.

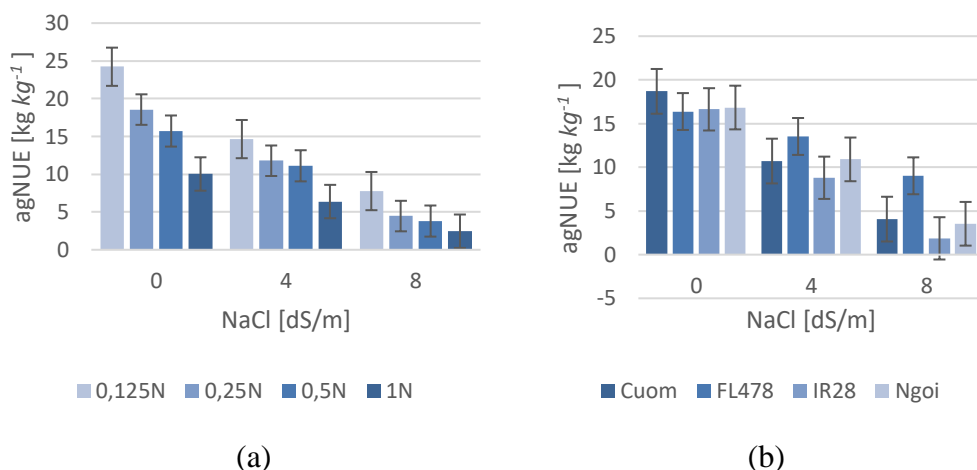


Figure 28: Agronomical NUE at active tillering: (a) in function of the salt concentration for the 4 N concentrations; (b) in function of the salt concentration for the 4 genotypes.

The complete GLM model was applied. Histogram: Least squares means. Error bar: 95% interval of confidence. Cultivar Cuom: trad. - tolerant; cultivar Ngoi: trad. - sensitive; cultivar FL478: improved - tolerant; cultivar IR28: improved - sensitive. 0,125N = 0.125g N; 0,25 = 0,25g N; 0,5N = 0,5g N; 1N = 1g N.

## Chapter V: Discussion and Perspectives

First of all, the effects of the genotype on the rice salt tolerance will be examined. Secondly, the results will be discussed to characterize the effects of nitrogen concentration on the rice growth and development in relation with salt tolerance. Thirdly, the Nitrogen Use Efficiency of rice as a function of salt tolerance will be discussed. In fourth place, the environmental conditions of the experiments will be discussed. Finally, possible perspectives on the subject will be proposed.

### 3 Discussion

#### 3.1 Effects of the Genotype on Rice Salt Tolerance

*Salt tolerance of cultivars was confirmed with  $Na^+/K^+$  ratio*

Cuom had the highest salt tolerance in comparison with the other tested cultivars. Then, Pokkali had a mitigate response to salinity, although it is a reference salt-tolerant variety. Finally, Ngoi was conformed to the expectation as a salt-sensitive cultivar. Those observations were clear on the basis of the dry shoot weight and could also be drawn according to the  $Na^+/K^+$  ratio (Figure 23 and Table 40). Cuom had a very low ratio (20% of Ngoi), Pokkali an intermediate one (50% of Ngoi) even though less significant and finally Ngoi had a high ratio. This 50% difference between salt-tolerant and salt sensitive cultivars is confirmed by the literature (Senadheera et al., 2009, Kavitha et al., 2012). The ratio shows the ability of the plant to exclude the  $Na^+$  or to reduce its uptake in case of salt stress. Salt-sensitive cultivars have a faster accumulation in their protoplasts than salt-tolerant ones. In addition, a depolarization of the root cells membrane under salt stress occurs in salt-sensitive varieties leading to a larger plasma membrane  $Na^+$  conductance (Kavitha et al., 2012). The low level of the  $Na^+$  concentration in cells is a main plant tolerance mechanism. Therefore, the toxicity of the  $Na^+$  ion is less marked for salt-tolerant cultivars.

Inconsistencies were found in the Vietnamese experiment regarding the global results of Ngoi. They were abnormally high in comparison of Cuom and FL478. The possible environmental reason is proposed in the subsection 1.4.

## 3.2 Effects of Nitrogen on Rice Salt Tolerance

### *Optimum Nitrogen concentration could decrease with the increasing salinity*

As for any nutrient, a nitrogen concentration has been calculated to be optimal for rice development in hydroponics (Yoshida et al., 1976). However, this has been calculated in the absence of abiotic stresses such as salinity. This optimum effect can be observed in absence of salt for the dry shoot weight. Nevertheless, the difference between the standard nitrogen concentration (2.86mM) and the 0.125N (0.358mM) is attenuated with the increasing salinity, leading to a similar result under strong salt conditions. This general attenuation and similar results under strong salinity can be observed for other variables such as the shoot length, the stomatal conductance, the chlorophyll content, the N content and the K content. Heymans (2017) showed that the optimum N concentration for the medium salinity level is half of the standard N (1.43mM). Under strong salinity, the results seem to be less influenced by the nitrogen concentration as they are similar to each other.

### *Salt sensitivity of rice occurred after emergence*

In the preliminary experiment on the germination stage, the results highlighted the absence of nitrogen concentration influence on the three-leaf stage samples, while only the salinity level and genotype significantly affected the rice germination. The salinity affected the rice just after the emergence, confirming the salt tolerance evolution described by Singh et al. (2009) and represented on the Figure 4. During the vegetative phase, the figure showed a high salt tolerance during germination, but it drops at the end of the first week and rice remains sensitive until the middle of the active tillering (7-8 weeks).

### *Plants subjected to salinity stress could have lower Nitrogen toxicity threshold*

NaCl toxicity would appear to be the major effect of the plant stress, covering up other influences. However, the nitrogen toxicity threshold could be lowered when a NaCl stress is present. It could be a hypothesis deserving consideration. Chen et al. (2013) showed that the shoot N concentration was lowered for a high/toxic N treatment (15mM) in comparison with a normal N treatment (2.86mM). This trend was highly correlated to the shoot biomass with a reduction of 27.3% under the high N treatment in comparison with the normal one. In addition, the low NUE cultivars were more sensitive to the N toxicity than the high NUE ones. In our experiment, the increasing NaCl concentration results in the decrease of the nitrogen content in shoot for the standard N, while an increasing content is observed for the low N concentration (Figure 16a). In addition, Cuom (high agNUE) had a higher N content than the two other

cultivars (low agNUE). The standard N could have reached the toxicity threshold and the effect can be observed with the decreasing shoot N content under salinity. On the contrary, the low N concentration seems to be a limiting factor and induces a positive effect. Therefore, the N toxicity threshold could be lowered in presence of NaCl stress.

*Salt tolerance mechanism could enhance protein synthesis under low N*

Nitrogen is a major compound of the photosynthetic machinery as mature chloroplasts contain 60% of the leaf N distributed mostly in the chlorophyll and Rubisco (50% of the protein content) (Morita, 1980; Lawlor, 2002). Therefore, the N uptake enhances the plant growth and the reverse influence is also true. A nitrogen deficiency decreases the soluble protein content and its synthesis per leaf. However, in our hydroponic experiment, for the 0.125N, the protein content is higher under medium salinity (Figure 14a), while it stays similar under the no salt condition and the strong salinity. This increase could be explained by a more important regeneration or synthesis of the Rubisco to increase the CO<sub>2</sub> fixation as it is a limiting molecule under salt stress (Adachi et al., 2017). Unfortunately, the strong salinity influences too negatively the rice growth to notice any effect.

### **3.3 Characterization of Nitrogen Use Efficiency for Rice Salt Tolerance**

Nitrogen Use Efficiency is directly linked to the dry weight and to the N applied or absorbed. The dry weight is a good overall indicator of the plant growth and gives a global result of the photosynthesis activity. As it is well known, nitrogen is a determinant element for the plant metabolic reactions (resulting i.e. in the osmoregulation) and its allocation inside the plant can lead to salt tolerance strategy. NUE allows a more specific way to understand the development of rice salt tolerance with information about possible N allocations. The absorption NUE (aNUE) shows the capacity of N absorption of rice, while the physiological NUE (pNUE) indicates the utilization of this absorbed N to synthesize organic matter – or dry weight. The agronomical NUE (agNUE) expresses the crop efficiency by the combination of the two previous indicators.

*Salt tolerance strategy could be determined with the NUE response of rice*

In the hydroponics experiment (Figure 19, Figure 20, Figure 21), all the different NUE showed that Pokkali and Ngoi behaved similarly. This observation could be explained by the possibility of a semi-tolerance of Ngoi and the suboptimal conditions for Pokkali cultivation (subsection 1.4.). This explanation could also be a reason of the Ngoi high results in the Vietnamese experiment. This hypothesis has to be taken

cautiously if referred to the Na/K ratio (subsection 1.1.). Moreover, the higher pNUE for the two cultivars may reflect a possible salt-tolerance strategy based on a biomass productivity, while Cuom could have a strategy preferentially based on the NaCl control which needs important amount of N (Figure 20b). The aNUE confirms the hypothetical strategy with high percentages for Cuom in general, but more specifically under medium salinity (Figure 19b).

### **3.4 Environmental Influences over the Experiments**

The environmental conditions were very different between the experiments in hydroponics and the one *in-situ* in Vietnam, resulting in high disparity of the plants' development.

In the phytotron, the environmental conditions could be not fully adapted for the cultivation of Pokkali. Indeed, this variety is photoperiod responsive and needs a higher light intensity to be productive. For those reasons, Pokkali is only cultivated during the summer season in Vietnam, when light intensity can reach 1000  $\mu\text{mol}/\text{m}^2\text{s}$  (5 times that of the phytotron intensity). It could be a possible argument of the unexpected results at UCL, while Pokkali is considered a reference salt-tolerant cultivar. Moreover, a fungus was spotted during germination on a part of Pokkali seeds, it could have caused a reduction of the growth rate. A disinfection treatment of the seeds could solve this problem.

In Vietnam, the weather was not optimal for rice cultivation as the experiment was conducted during the spring season. Indeed, the temperatures were quite low, and the sowing had to be delayed one month. Moreover, the light intensity was also low with an important cloud cover. All these factors could influence the growth rate and therefore the salt tolerance of the plants, as rice is more sensitive to salinity during the early seedling period. However, the calendar was adapted to the physiological stages, so the results should not be biased by the temperature and light intensity. The main difficulty was to maintain stable the salinity conditions. The EC was checked and adjusted at least twice a week but when heavy rains occurred, this process had to be done again. After a heavy rain, the EC of the supposed strong salinity could diminish to the medium one. Unfortunately, the stabilization of a pot salinity could only be observed one day after the adjustment, hence the salt level being not perfectly constant. Fortunately, only a few heavy rains were problematic during the third week of treatment. The rainfall problem was solved just after sampling the active tillering with the relocation of the experiment inside a covered nethouse.

## 4 Perspectives

First of all, adapted analysis could be conducted on the root system as it constitutes the first plant system in time to be exposed to the salt stress. The quantification of the variables for the roots should be interesting to confirm similar trends or highlight different ones influencing the salt tolerance (i.e. Na, K, N) (Shen et al., 2013). Moreover, taking into account the totality of the plant should be more accurate for analysis such as the NUE. Shoot/root ratio can also bring valuable information to see if the translocation is influenced by the combined factors.

The translocation and compartmentalization of toxic elements, such as  $\text{Na}^+$ , is considered a salt-tolerant mechanism at the plant level. Therefore, the separation of the old and young leaves could highlight different interactions between N and NaCl.

The implementation of additional salinities, especially between the medium and strong salinity, could make the study of interaction between N and NaCl more accurate, before the salinity effect covers totally any other interaction. For the same reason, additional N concentration could be implemented in order to highlight information between variables or on the threshold N level interacting with salinity. It has been shown that the N source has an effect on the plant growth under saline conditions (Leidi et al., 1991; Nhung et al., 2017).

An analysis of the  $\text{Cl}^-$  content adapted to the rice conditions could complete the study on salt tolerance. The Cl toxicity is usually considered less damaging to the plant than  $\text{Na}^+$  and therefore usually put aside. However, the Cl analysis could identify another response pattern for the tolerance mechanism.

Our study intended to quantify the chloride content but the low contents in dry matter and the limited dry matter per sample were the limiting factors for the technical analysis. This problematic was also present for the analysis of the elements in roots. A solution could consist to the increase of the repetition number and then join samples together in order to realize analysis requiring bigger amount of material.

Regarding the Vietnamese experiment, the lack of photosynthetic data under strong salinity prevents a complete analysis. Additional repetitions could eventually solve the problem.

Always for the *in-situ* experiment, the study could be completed with more physiological variables analysis. Minerals, proteins and chlorophyll contents could point out links between hydroponics and the pot experiments.

The experimental conditions in Vietnam could have been better controlled with the direct implementation of the rice inside a covered nethouse to avoid the perturbing effect of the precipitations on the salt levels. In order to insure optimal environmental conditions (temperature, light intensity ...), the experiment could take place during the summer rice season.

Finally, rice field soils could be analyzed in order to manage a more accurate fertilization and to decrease N fertilization in case of saline conditions.

## Chapter VI: Conclusions

The **main objective** of this thesis was to analyze the influence of rice (*Oryza sativa*) genotype over its Nitrogen Use Efficiency (NUE) under salinity stress. The results showed conclusive interactions between NUE and the rice salt tolerance mechanisms. The experiments in hydroponics, evaluating the interactions between nitrogen concentration, genotype and salinity levels during the vegetative phase, allowed to identify trends on the studied morphological or physiological variables. Several conclusions are proposed below.

The **first specific objective** was to study and highlight trends of the relation between the three fixed parameters (genotype, salinity and nitrogen concentration), by performing an ANOVA analysis of different variables. A first observation showed that the salt sensitivity began just after the seedling emergence.

The  $\text{Na}^+/\text{K}^+$  ratio confirmed the salt-tolerance efficiency for each genotype. However, Pokkali showed an intermediate ratio between the low value of Cuom and the high value of Ngoi. Therefore, the reintroduction of Cuom cultivar could be interesting for fields encountering saline conditions.

The non-optimal standard nitrogen concentration (2.86 mM) for rice hydroponics under salt stress was confirmed by the experiments. Indeed, the difference between the standard N and the low N (0.358 mM) concentrations regarding the dry shoot weight was attenuated while salinity increased, until reaching similar values under strong salinity (113mM). Therefore, the N optimum concentration seems to decrease with the increasing salinity. That conclusion was confirmed by Heymans (2017) with an optimum N concentration of 1.43mM for the medium salinity (56 mM). The results showed a possible interaction between the NaCl and the N toxicity. The N toxicity threshold could be lowered for a higher N concentration under saline conditions.

The hydroponics results indicated an influence of the rice salt tolerance on the NUE, confirming the salt tolerance of the cultivars. Cuom presented the best results for the absorption NUE (aNUE) and the agronomical NUE (agNUE). Pokkali and Ngoi had similar low results for the aNUE and agNUE. However, they had a higher physiological NUE (pNUE) when the salt stress was present.

The **second specific objective** was to understand some physiological patterns and plant responses associated with those trends. The results of the pNUE allowed to point out a possible different strategy of Cuom in comparison with the two other cultivars. Indeed, Cuom had a low pNUE, while its aNUE was high, suggesting a utilization of the nitrogen to synthesize costly-N compounds in order to limit the NaCl damage. On the

other side, the two other cultivars could focus in the biomass production as the aNUE and agNUE were low but the pNUE was higher than for Cuom. This hypothesis is consolidated with the  $\text{Na}^+/\text{K}^+$  ratio of Cuom. However, the ratio difference between Pokkali and Ngoi could raise doubts regarding this result. This hypothesis could lead to a selection of salt-tolerant cultivars with a high aNUE that will synthesize N-compounds for its tolerance strategy.

The *in-situ* experiment in Vietnam did not allow us to confirm or invalidate the trends and hypotheses highlighted by the hydroponics experiments. The environmental conditions and the low survival rate under the strong salinity caused a too important lack of data to conduct a comparative analysis.

In conclusion, the reduction of nitrogen fertilization under saline conditions could help the rice development. Indeed, overuse of N should be avoided in general, but especially under saline conditions in order to decrease the possible higher risk of nitrogen toxicity. The utilization of selected salt-tolerant cultivars with a high aNUE could improve the rice cultivation under NaCl stress. Therefore, rice cultivation could be possibly maintained in potential saline soils.

## Chapter VII: References

- Abdelgadir, E.M., Oka, M., Fujiyama, H., 2005. Nitrogen nutrition of rice plants under salinity. *Biologia Plantarum* 49, 99–104. <https://doi.org/10.1007/s10535-005-0104-8>
- Adachi, S., Yoshikawa, K., Yamanouchi, U., Tanabata, T., Sun, J., Ookawa, T., Yamamoto, T., Sage, R. F., Hirasawa, T., and Yonemaru, J. (2017). Fine mapping of carbon assimilation rate 8, a quantitative trait locus for flag leaf nitrogen content, stomatal conductance and photosynthesis in rice. *Frontiers in plant science*, 8.
- Ali, Y., Aslam, Z., Ashraf, M.Y., Tahir, G.R., 2004. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *International Journal of Environmental Science & Technology* 1, 221–225. <https://doi.org/10.1007/BF03325836>
- Ashraf, M., Shahzad, S.M., Imtiaz, M., Rizwan, M.S., Arif, M.S., Kausar, R., 2018. Nitrogen nutrition and adaptation of glycophytes to saline environment: a review. *Archives of Agronomy and Soil Science* 64, 1181–1206. <https://doi.org/10.1080/03650340.2017.1419571>
- Aulakh, M., Khera, T., Doran, J., Bronson, K., 2001. Denitrification, N<sub>2</sub>O and CO<sub>2</sub> fluxes in rice-wheat cropping system as affected by crop residues, fertilizer N and legume green manure. *Biology and Fertility of Soils* 34, 375–389. <https://doi.org/10.1007/s003740100420>
- Bernhard, A., 2010. The Nitrogen cycle: Processes, Players, and human impact. 2, *Nature Education Knowledge* 2, 9.
- Bijay-Singh, Singh, V.K., 2017. Fertilizer management in rice, in: *Rice Production Worldwide*. Chauhan B.S., Jabran, K., Mahajan, G., p. 563. DOI 10.1007/978-3-319-47516-5\_10
- Bollich, P.K., Lindau, C.W., Norman, R.J., 1994. Management of fertilizer nitrogen in dry-seeded, delayed-flood rice. *Aust. J. Exp. Agric.* 34, 1007–1012. <https://doi.org/10.1071/ea9941007>
- Bouman, B.A., Tuong, T., 2001. Field water management to save water and increase its productivity in irrigated lowland rice. *Agricultural Water Management* 49, 11–30. [https://doi.org/10.1016/S0378-3774\(00\)00128-1](https://doi.org/10.1016/S0378-3774(00)00128-1)
- Buresh, R.J., Reddy, K.R., van Kessel, C., 2008. Nitrogen Transformations in Submerged Soils. *Nitrogen in Agricultural Systems agronomy monogra*, 401–436. <https://doi.org/10.2134/agronmonogr49.c11>
- Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. *AMBIO: A Journal of the Human Environment* 31, 132–140. <https://doi.org/10.1579/0044-7447-31.2.132>
- Cassman, K.G., Kropff, M.J., Gaunt, J., Peng, S., 1993. Nitrogen use efficiency of rice reconsidered: What are the key constraints?, in: *Plant Nutrition — from Genetic Engineering to Field Practice, Developments in Plant and Soil Sciences*. Springer, Dordrecht, pp. 471–474. [https://doi.org/10.1007/978-94-011-1880-4\\_99](https://doi.org/10.1007/978-94-011-1880-4_99)
- Chauhan, B.S., Jabran, K., Mahajan (eds.), G., 2017. *Rice Production Worldwide*, 1st ed. Springer International Publishing.
- Chauhan, B.S., Prabhjyot-Kaur, Mahajan, G., Randhawa, R.K., Singh, H., Kang, M.S., 2014. Global Warming and Its Possible Impact on Agriculture in India, in: *Advances in Agronomy*. Elsevier, pp. 65–121. <https://doi.org/10.1016/B978-0-12-420225-2.00002-9>

- Chen, G., Guo, S., Kronzucker, H.J., Shi, W. 2013. Nitrogen Use Efficiency (NUE) in rice links to NH<sub>4</sub><sup>+</sup> toxicity and futile NH<sub>4</sub><sup>+</sup> cycling in roots). *Plant and Soil* 369, 351-363.  
<https://doi.org/10.1007/s11104-012-1575-y>
- De Datta, S.K., 1981. Principles and practices of rice production / Surajit K. De Datta, John Wiley & Sons. ed.
- De Datta, S.K., Obcemea, W.N., Real, J.G., Trevitt, A.C.F., Freney, J.R., Simpson, J.R., 1989. Measuring Nitrogen Losses from Lowland Rice Using Bulk Aerodynamic and Nitrogen-15 Balance Methods. *Soil Science Society of America Journal* 53, 1275–1281.  
<https://doi.org/10.2136/sssaj1989.03615995005300040047x>
- De Datta, S.K., Samson, M.I., Obcemea, W.N., Real, J.G., Buresh, R.J., 1991. Direct Measurement of Ammonia and Denitrification Fluxes from Urea Applied to Rice. *Soil Science Society of America Journal* 55, 543. <https://doi.org/10.2136/sssaj1991.03615995005500020043x>
- Delmo-Organo, N., Delfin, E.F., Gregorio, G.B., Lantican, N.B., Simbahan, J.F., Paterno, E.S., 2017. ASSOCIATED WITH RICE (*Oryza sativa* L.) 23, 12.
- Denman, K.L., Chidthaisong, A., Heinze, C., Ramachandran, S., Archer, D., Bousquet, P., Easterling, D., Gong, S., Jones, A., Leck, C., Peylin, P., Riebesell, U., Schulz, M., Noije, T.V., Boonpragob, K., 2007. Couplings Between Changes in the Climate System and Biogeochemistry, in: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 90.
- Dobermann, A., Witt, C., Dawe, D., Abdulrachman, S., Gines, H., Nagarajan, R., Satawathanont, S., Son, T., Tan, P., Wang, G., Chien, N., Thoa, V.T., Phung, C., Stalin, P., Muthukrishnan, P., Ravi, V., Babu, M., Chatuporn, S., Sookthongsa, J., Sun, Q., Fu, R., Simbahan, G., Adviento, M.A., 2002. Site-specific nutrient management for intensive rice cropping systems in Asia. *Field Crops Research* 74, 37–66. [https://doi.org/10.1016/S0378-4290\(01\)00197-6](https://doi.org/10.1016/S0378-4290(01)00197-6)
- Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C<sub>3</sub> plants. *Oecologia* 78, 9–19.  
<https://doi.org/10.1007/BF00377192>
- Fageria, N.K., 2008. *The Use of Nutrients in Crop Plants*, 1st ed. CRC Press, pp.430.
- Fageria, N.K., 2007. Yield Physiology of Rice. *Journal of Plant Nutrition* 30, 843–879.  
<https://doi.org/10.1080/15226510701374831>
- Fageria, N.K., Baligar, V.C., Heinemann, A.B., Carvalho, M.C.S., 2015. Nitrogen Uptake and Use Efficiency in Rice, in: *Nutrient Use Efficiency: From Basics to Advances*. Springer, New Delhi, pp. 285–296.  
[https://doi.org/10.1007/978-81-322-2169-2\\_18](https://doi.org/10.1007/978-81-322-2169-2_18)
- Gao, J.-P., Chao, D.-Y., Lin, H.-X., 2007. Understanding Abiotic Stress Tolerance Mechanisms: Recent Studies on Stress Response in Rice. *Journal of Integrative Plant Biology* 49, 742–750.  
<https://doi.org/10.1111/j.1744-7909.2007.00495.x>
- Gaudin, R., Dupuy, J., 1999. Ammoniacal Nutrition of Transplanted Rice Fertilized with Large Urea Granules. *Agronomy Journal* 91, 33–36.  
<https://doi.org/10.2134/agronj1999.00021962009100010006x>
- Global Rice Science Partnership, 2013. *Rice almanac: source book for the most important economic activities on Earth*, Fourth Edition. ed. IRRI, Los Baños, Philippines.
- Gregorio, G.B., Senadhira, D., 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 86–86. <https://doi.org/10.1007/BF00222098>

- Gregorio, G.B., Senadhira, D., Mendoza, R.D., 1997. Screening rice for salinity tolerance. IRRI DISCUSSIONPAPER SERIES 31.
- Gregorio, G.B., Senadhira, D., Mendoza, R.D., Manigbas, N.L., Roxas, J.P., Guerta, C.Q., 2002. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crops Research* 76, 91–101. [https://doi.org/10.1016/S0378-4290\(02\)00031-X](https://doi.org/10.1016/S0378-4290(02)00031-X)
- Hairmansis, A., Berger, B., Tester, M., Roy, S.J., 2014. Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. *Rice* 7. <https://doi.org/10.1186/s12284-014-0016-3>
- Hameed, A., Qadri, T.N., Azooz, M.M., Ahmad, P., 2013. Salt Tolerance in Rice: Present Scenario and Future Prospects, in: *Ecophysiology and Responses of Plants under Salt Stress*. Springer, New York, NY, pp. 203–211. [https://doi.org/10.1007/978-1-4614-4747-4\\_7](https://doi.org/10.1007/978-1-4614-4747-4_7)
- Hasegawa, P.M., Bressan, R.A., Zhu, J.-K., Bohnert, H.J., 2003. Annual Review of Plant Physiology and Plant Molecular Biology 51, 463–499. <https://doi.org/10.1146/annurev.arplant.51.1.463>
- Hussain, M., Ahmad, S., Hussain, S., Lal, R., Ul-Allah, S., Nawaz, A., 2018. Rice in Saline Soils: Physiology, Biochemistry, Genetics, and Management, in: *Advances in Agronomy*. Elsevier, pp. 231–287. <https://doi.org/10.1016/bs.agron.2017.11.002>
- IRRI, n.d. Ricepedia [WWW Document]. Ricepedia. URL <http://ricepedia.org/> (accessed 7.30.18a).
- IRRI, n.d. Water management - IRRI Rice Knowledge Bank [WWW Document]. Rice Knowledge Bank. URL <http://www.knowledgebank.irri.org/step-by-step-production/growth/water-management> (accessed 7.18.18b).
- Kajala, K., Covshoff, S., Karki, S., Woodfield, H., Tolley, B.J., Dionora, M.J.A., Mogul, R.T., Mabilangan, A.E., Danila, F.R., Hibberd, J.M., Quick, W.P., 2011. Strategies for engineering a two-celled C4 photosynthetic pathway into rice. *Journal of Experimental Botany* 62, 3001–3010. <https://doi.org/10.1093/jxb/err022>
- Kavitha, P.G., Miller, A.J., Mathew, M.K., Maathuis, F.J.M., 2012. Rice cultivars with differing salt tolerance contain similar cation channels in their root cells. *Journal of Experimental Botany* 63, 3289–3296. <https://doi.org/10.1093/jxb/ers052>
- Kijne, J.W., Barker, R., Molden, D., 2003. Water Productivity in Agriculture: Limits and Opportunities for Improvement (Comprehensive Assessment of Water Management in Agriculture Series, 1), First ed.
- Kumar, A., Dixit, S., Ram, T., Yadaw, R.B., Mishra, K.K., Mandal, N.P., 2014. Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. *J Exp Bot* 65, 6265–6278. <https://doi.org/10.1093/jxb/eru363>
- Lawlor, D.W., 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany* 53, 773–787. <https://doi.org/10.1093/jxb/53.370.773>
- Lee, K.-S., Choi, W.-Y., Ko, J.-C., Kim, T.-S., Gregorio, G.B., 2003. Salinity tolerance of japonica and indica rice (*Oryza sativa* L.) at the seedling stage. *Planta* 216, 1043–1046. <https://doi.org/10.1007/s00425-002-0958-3>
- Leidi, E.O., Silberbush, M., Lips, S.H., 1991a. Wheat growth as affected by nitrogen type, pH and salinity. I. biomass production and mineral composition. *Journal of Plant Nutrition* 14, 235–246. <https://doi.org/10.1080/01904169109364198>
- Li, H., Hu, B., Chu, C., 2017. Nitrogen use efficiency in crops: lessons from Arabidopsis and rice. *Journal of Experimental Botany* 68, 2477–2488. <https://doi.org/10.1093/jxb/erx101>

- Li, P., Lu, J., Wang, Y., Wang, S., Hussain, S., Ren, T., Cong, R., Li, X., 2018. Nitrogen losses, use efficiency, and productivity of early rice under controlled-release urea. *Agriculture, Ecosystems & Environment* 251, 78–87. <https://doi.org/10.1016/j.agee.2017.09.020>
- Li, Y., Yang, X., Ren, B., Shen, Q., Guo, S., 2012. Why Nitrogen Use Efficiency Decreases Under High Nitrogen Supply in Rice (*Oryza sativa* L.) Seedlings. *Journal of Plant Growth Regulation* 31, 47–52. <https://doi.org/10.1007/s00344-011-9218-8>
- Linh, L.H., Linh, T.H., Xuan, T.D., Ham, L.H., Ismail, A.M., Khanh, T.D., 2012. Molecular Breeding to Improve Salt Tolerance of Rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *International Journal of Plant Genomics* 2012, 1–9. <https://doi.org/10.1155/2012/949038>
- Linquist, B.A., Liu, L., van Kessel, C., van Groenigen, K.J., 2013. Enhanced efficiency nitrogen fertilizers for rice systems: Meta-analysis of yield and nitrogen uptake. *Field Crops Research* 154, 246–254. <https://doi.org/10.1016/j.fcr.2013.08.014>
- Lutts, S., Kinet, J.M., Bouharmont, J., 1996. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regulation* 19, 207–218. <https://doi.org/10.1007/BF00037793>
- Lutts, S., Kinet, J.M., Bouharmont, J., 1996. NaCl-induced Senescence in Leaves of Rice (*Oryza sativa* L.) Cultivars Differing in Salinity Resistance. *Annals of Botany* 78, 389–398. <https://doi.org/10.1006/anbo.1996.0134>
- Lutts, S., Majerus, V., Kinet, J.-M., 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiologia Plantarum* 105, 450–458. <https://doi.org/10.1034/j.1399-3054.1999.105309.x>
- Mae, T., 1997. Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant and Soil* 196, 201–210. <https://doi.org/10.1023/A:1004293706242>
- Mohammadi-Nejad, G., Singh, R.K., Arzani, A., Rezaie, A.M., Sabouri, H., Gregorio, G.B., 2010. Evaluation of salinity tolerance in rice genotypes. *International Journal of Plant Production* 10.
- Molden, D., Oweis, T., Steduto, P., Bindraban, P., Hanjra, M.A., Kijne, J., 2010. Improving agricultural water productivity: Between optimism and caution. *Agricultural Water Management* 97, 528–535. <https://doi.org/10.1016/j.agwat.2009.03.023>
- Nguyen, Y.T.B., Kamoshita, A., Dinh, V.T.H., Matsuda, H., Kurokura, H., 2017. Salinity intrusion and rice production in Red River Delta under changing climate conditions. *Paddy and Water Environment* 15, 37–48. <https://doi.org/10.1007/s10333-016-0526-2>
- Nhung, P., Hanh, T., Bertin, P., Cuong, P., 2017. Effect of inorganic nitrogen forms and concentrations on growth of rice genotypes under severe saline condition. *Vietnam J Agri Sci* 189–197.
- Food and Agriculture Organization of the United Nations (FAO), n.d. FAOSTAT [WWW Document]. URL <http://www.fao.org/faostat/> (accessed 6.30.18).
- Parihar, P., Singh, S., Singh, R., Pratap Singh, V., Prasad, S.M., 2015. Effect of salinity stress on plants and its tolerance strategies: a review. *Environ Sci Pollut Res* 22, 4056–4075. <https://doi.org/DOI10.1007/s11356-014-3739-1>
- Paul, B.K., Rashid, H., 2017. Chapter Five - Salinity Intrusion and Impacts, in: Paul, B.K., Rashid, H. (Eds.), *Climatic Hazards in Coastal Bangladesh*. Butterworth-Heinemann, Boston, pp. 153–182. <https://doi.org/10.1016/B978-0-12-805276-1.00005-3>
- Paul, D., Lade, H., 2014. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. *Agronomy for Sustainable Development* 34, 737–752. <https://doi.org/10.1007/s13593-014-0233-6>

- Prabhjyot-Kaur, Hundal, S., 2006. Effect of possible futuristic climate change scenarios on productivity of some kharif and rabi crops in the central agro-climatic zone of Punjab. *J Agric Phys* 21–27.
- Prasad, R., Singh Shivay, Y., Kumar, D., 2017. Current Status, Challenges, and Opportunities in Rice Production, in: *Rice Production Worldwide*. Chauhan B.S., Jabran, K., Mahajan, G., p. 563.
- Quynh-Hoa, P., Xuan-An, T., Thi-Nha-Trang, N., Thi-Thuy-Anh, T., Hai-Yen, H., Thi-Hong-Van, N., Thi-Hanh, T., Thi-Phuc, D., 2016. Investigation of Polymorphisms in Coding Region of OsHKT1 in Relation to Salinity in Rice. *Rice Science* 23, 334–338. <https://doi.org/10.1016/j.rsci.2016.05.004>
- Rahman, S., Matsumuro, T., Miyake, H., Takeoka, Y., 2001. Effects of Salinity Stress on the Seminal Root Tip Ultrastructures of Rice Seedlings (*Oryza sativa* L.). *Plant Production Science* 4, 103–111. <https://doi.org/10.1626/pps.4.103>
- Rakshit, A., Singh, H.B., Sen (eds.), A., 2015. *Nutrient Use Efficiency: from Basics to Advances*, 1st ed. Springer India.
- Raun, W.R., Johnson, G.V., 1999. Improving Nitrogen Use Efficiency for Cereal Production. *Agronomy Journal* 91, 357. <https://doi.org/10.2134/agronj1999.00021962009100030001x>
- Raven, J.A., 1985. Tansley review No. 2. Regulation of pH and generation of osmolarity in vascular plants: a cost-benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytologist* 101, 25–77. <https://doi.org/10.1111/j.1469-8137.1985.tb02816.x>
- Rengasamy, P., 2006. World salinization with emphasis on Australia. *Journal of Experimental Botany* 57, 1017–1023. <https://doi.org/10.1093/jxb/erj108>
- Salsac, L., Chaillou, S., Morot Gaudry, J.F., Lesaint, C., Jolivet, E., 1987. Nitrate and ammonium nutrition in plants [organic anion, ion accumulation, osmolarity]. *Plant Physiology and Biochemistry*.
- Senguttuvel, P., Vijayalakshmi, C., Thiyagarajan, K., Kannanbapu, J.R., Kota, S., Padmavathi, G., Geetha, S., Sritharan, N., Viraktamath, B.C., 2014. Changes in photosynthesis, chlorophyll fluorescence, gas exchange parameters and osmotic potential to salt stress during early seedling stage in rice (*Oryza sativa* L.). *Sabrao Journal of Breeding & Genetics* 46, 120–135.
- Shaviv, A., 2001. Advances in controlled-release fertilizers, in: *Advances in Agronomy*. Elsevier, pp. 1–49. [https://doi.org/10.1016/S0065-2113\(01\)71011-5](https://doi.org/10.1016/S0065-2113(01)71011-5)
- Shi, W.M., Xu, W.F., Li, S.M., Zhao, X.Q., Dong, G.Q., 2010. Responses of two rice cultivars differing in seedling-stage nitrogen use efficiency to growth under low-nitrogen conditions. *Plant Soil* 326, 291. <https://doi.org/10.1007/s11104-009-0007-0>
- Singh, B., Singh, V., 2017. Fertilizer Management in Rice, in: *Rice Production Worldwide*. pp. 217–253. [https://doi.org/10.1007/978-3-319-47516-5\\_10](https://doi.org/10.1007/978-3-319-47516-5_10)
- Singh, R.K., Redoña, E., Refuerzo, L., 2009. Varietal Improvement for Abiotic Stress Tolerance in Crop Plants: Special Reference to Salinity in Rice, in: Pareek, A., Sopory, S.K., Bohnert, H.J. (Eds.), *Abiotic Stress Adaptation in Plants*. Springer Netherlands, Dordrecht, pp. 387–415. [https://doi.org/10.1007/978-90-481-3112-9\\_18](https://doi.org/10.1007/978-90-481-3112-9_18)
- Takenaga, H., 1995. Science of rice plant: Physiology, in: *Internal Factors in Relation to Nutrient Absorption*. Matsuo T, Kumazawa K, Ishii R, Ishihara K, Hirata H, Tokyo, pp. 294–309.
- Takeuchi, K., Saito, O., Matsuda, H., Mohan (eds.), G., 2018. *Resilient Asia: Fusion of Traditional and Modern Systems for a Sustainable Future*, 1st ed, Science for Sustainable Societies. Springer Japan.
- Tester, M., 2003. Na<sup>+</sup> Tolerance and Na<sup>+</sup> Transport in Higher Plants. *Annals of Botany* 91, 503–527. <https://doi.org/10.1093/aob/mcg058>

- Timmer, C.P., 2010. The Changing Role of Rice in Asia's Food Security. 15, ADB Sustainable Development Working Paper Series 24.
- Tran, T., Nguyen, X.H., Huynh, T.L.H., Tran, V.T., Duong, N.T., Doan, T.T.H., 2017. Estimating sea level rise for Vietnam East Sea. *Vietnam Journal of Science, Technology and Engineering* 59, 73–78.
- Tuong, T., Bouman, B.A., 2003. Rice production in water-scarce environments, in: *Water Productivity in Agriculture: Limits and Opportunities for Improvements*. Kijne JW, Barker R, Molden D, Cambridge, p. 320.
- Vijayalakshmi, D., Kishor Ashok, S., Raveendran, M., 2014. Screening for salinity stress tolerance in rice and finger millet genotypes using shoot Na<sup>+</sup>/K<sup>+</sup> ratio and leaf carbohydrate contents as key physiological traits. *Indian Journal of Plant Physiology* 19, 156–160. <https://doi.org/10.1007/s40502-014-0090-y>
- Vijayalakshmi, P., Kiran, T.V., Rao, Y.V., Srikanth, B., Rao, I.S., Sailaja, B., Surekha, K., Rao, P.R., Subrahmanyam, D., Neeraja, C.N., Voleti, S.R., 2013. Physiological approaches for increasing nitrogen use efficiency in rice. *Indian Journal of Plant Physiology* 18, 208–222. <https://doi.org/10.1007/s40502-013-0042-y>
- Wassmann, R., Dobermann, A., 2007. Climate Change Adaptation through Rice Production in Regions with High Poverty Levels. *SAT eJournal* 4, 24.
- Weller, S., Kraus, D., Ayag, K.R.P., Wassmann, R., Alberto, M.C.R., Butterbach-Bahl, K., Kiese, R., 2015. Methane and nitrous oxide emissions from rice and maize production in diversified rice cropping systems. *Nutrient Cycling in Agroecosystems* 101, 37–53. <https://doi.org/10.1007/s10705-014-9658-1>
- Wen, F., Zhang, Z., Bai, T., Xu, Q., Pan, Y., 2010. Proteomics reveals the effects of gibberellic acid (GA<sub>3</sub>) on salt-stressed rice (*Oryza sativa* L.) shoots. *Plant Science* 178, 170–175. <https://doi.org/10.1016/j.plantsci.2009.11.006>
- Wilson, C.E., Wells, B.R., Norman, R.J., 1994. Fertilizer Nitrogen Uptake by Rice from Urea-Ammonium Nitrate Solution vs. Granular Urea. *Soil Science Society of America Journal* 58, 1825. <https://doi.org/10.2136/sssaj1994.03615995005800060035x>
- Workman, D., 2018. Rice Exports by Country [WWW Document]. World's Top Exports. URL <http://www.worldstopexports.com/rice-exports-country/> (accessed 7.16.18).
- Wu, H., 2018. Plant salt tolerance and Na<sup>+</sup> sensing and transport. *The Crop Journal* 6, 215–225. <https://doi.org/10.1016/j.cj.2018.01.003>
- Ye, C., Tenorio, F.A., Argayoso, M.A., Laza, M.A., Koh, H.-J., Redoña, E.D., Jagadish, K.S., Gregorio, G.B., 2015. Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics* 16. <https://doi.org/10.1186/s12863-015-0199-7>
- Yoshida, S., 1981. *Fundamentals of Rice Crop Science*. Int. Rice Res. Inst.
- Yoshida, S., Forno, D., Cock, J., Gomez, K., 1976. *Laboratory manual for physiological studies of rice*, Third Edition. ed. International Rice Research Institute, Philippines (IRRI).
- Zafar, S.A., Shokat, S., Ahmed, H.G.M.-D., Khan, A., Ali, M.Z., Atif, R.M., 2015. Assessment of salinity tolerance in rice using seedling based morpho-physiological indices. *Advancements in Life Sciences* 2, 142–149.
- Zhu, J.-K., 2001. Plant salt tolerance. *Trends in Plant Science* 6, 66–71. [https://doi.org/10.1016/S1360-1385\(00\)01838-0](https://doi.org/10.1016/S1360-1385(00)01838-0)
- Zhu, Z.L., Chen, D.L., 2002. Nitrogen fertilizer use in China – Contributions to food production, impacts on the environment and best management strategies. *Nutrient Cycling in Agroecosystems* 63, 117–127. <https://doi.org/10.1023/A:1021107026067>



# Evaluation of Nitrogen Use Efficiency (NUE) of rice (*O. sativa*) under saline conditions

Submitted by Mathieu Bonnavé

**Abstract** Rice (*Oryza sativa* L.) is a major food supply in the world and many challenges for its cultivation sustainability have to be faced. One of these challenges consists in an efficient management of the nitrogen fertilization. Indeed, nitrogen is one of the most important elements for the plant growth and development. However, rice cropping conditions can be favorable to nitrogen losses leading to problems like eutrophication. Developing varieties reaching higher nitrogen use efficiency could represent a valuable improvement for the crop yield and economically, but also a sustainable improvement. Another challenge is to deal with the increasing risk of abiotic stress due to climate change, such as salinity which is more frequently present in Vietnamese deltas.

This study consisted in an analysis of the interactions between three fixed parameters: the nitrogen fertilization applied, the salinity level and the rice genotype. Two first experiments were conducted in hydroponics during the early vegetative phase with two nitrogen concentrations (standard N and 0.125N) and three increasing salt levels (no salt, moderate and strong salinity) for three genotypes Cuom: Vietnamese salt tolerant; Pokkali: salt tolerant reference; Ngoi: Vietnamese salt sensitive). The study was completed with an experiment in pots in Vietnam to validate the findings of the hydroponics analysis. This last experiment was conducted until the harvest but was focused on the active tillering.

Several conclusions can be highlighted with this work. The first one was the absence of salt sensitivity before the seedling emergence. The second conclusion showed that the N toxicity seemed to increase with the salinity. Finally, the salt tolerance had an influence on the Nitrogen Use Efficiency (NUE). A high salt-tolerance cultivar, such as Cuom, presented a high absorption (aNUE) and agronomical NUE (agNUE) coupled to a low physiological one (pNUE), while the opposite NUE levels were more related to salt-sensitive cultivar. The Vietnamese in-situ experiment did not invalidate the conclusions but can only confirm the negative influence of salt stress on rice and the basic nitrogen effects in absence of salt. Nitrogen Use Efficiency is a valuable indicator to be considered in future studies regarding the salinity tolerance of rice.