REPUBLIC OF TUNISIA

MINISTRY OF AGRICULTURE, HYDRAULIC RESOURCES AND FISHERIES

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
CARTHAGE UNIVERSITY

NATIONAL AGRONOMIC INSTITUTE OF TUNISIA

Doctoral School Sciences and Techniques of Agronomy and Environment

Doctoral Thesis in Agronomic Sciences

Specialty: Plant Production Sciences

Nitrogen use efficiency (NUE) components and plasticity of nitrogen metabolism in barley (*Hordeum vulgare* L.) under saline stress

Presented by

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Tunis, 2021

Dedication

Thank you GOD for giving me the strength and the determination to complete this work.

I would like dedicate this work to:

my family

A special feeling of gratitude to the most precious gif of GOD, my loving parents, Abdelkadeur and Fatma, your words of encouragement and push for tenacity ring in my ears. Thank you for all the sacrifices you made for me. Thank you for the values you teach me. Thank you for making me the strong woman I am today.

I also dedicate this work to my kind sisters Imen and Sana, my dear brothers Nidhal and Salaheddine, and my special young brother Samed, they have never left my side. To my wonderful innocent Nourhen, the source of my happiness and positivity. Thank you all for your love and unconditional care.

& my friends

I dedicate this work to my friends Souad, Sihem, Basma M., Besma B.S., Hayfa, who surround me, and give me energy and inspiration. Thank you for your warm environment you provided for me.

A special dedication to Zied, thank you for your encouragements and unlimited support, I will always appreciate all you have done for me to achieve my Goals. Thank you for being there for me!

Thank you for your believe in me. Thank you because you are in my life. I love you...

Zahra

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Acknowledgment

I am very grateful to my supervisor **Prof. Youssef Trifa** for his unfailing guidance and immense knowledge that helped me and taught me a lot. I highly appreciate all the valuable discussions we had during the Ph.D. period about my experiments and their outcomes. I don't think I have enough words to thank him for his unlimited support.

Special thanks to my co-supervisor, **Dr. Sawssen Ayadi Kallel** for her advice and guidance through these years. She was more than generous with her expertise and precious time. I thank her for her patience, kindness, encouragement and generosity to provide support. Her excitement and willingness to provide comments and feedback made the completion of this research an agreeable experience.

My sincere thanks go to **Prof. Sergio Esposito** my supervisor in Italy, for giving me the opportunity to join his wonderful team and conduct a part of my research in his laboratory. I thank him for his incredible support during my research project. and for his patience throughout the publication process. His contribution, made possible the completion of this work. I thank him for his humanity; I will never forget what he did to make some difficult times easier.

I also address my thanks to **Prof. Abdelaziz Mougou**. It is a great honor to have him asthe president of my thesis committee.

I also thank **Dr. Mohamed Annabi** to have accepted to go throughout my thesis document for its evaluation. I thank him for his time spent for this purpose.

I would like also to think **Dr. Slim Slim** for his countless hours of reflecting and reading, and for the time and expertise in evaluating my work.

My thanks go also to **Prof. Guider Tibaoui** for accepting to be the examiner of my manuscript. I thank him for his precious time and expertise.

My sincerest gratitude to **Prof. Hajer Amara** for accepting me in her laboratory "Genetics and Plant breeding" at the National Institute of Agronomy of Tunisia and for giving me the opportunity to conduct my experiments. I also address my sincere thanks to all the staff of our laboratory for their help and collaboration.

I thank my colleague **Dr. Zoubeir Chamekh** for his precious help in conducting the field experiment.

I would like to express my deepest thanks and appreciation to my colleague and friend **Dr. Zied Hammami** for sharing his knowledge and expertise in statistical analysis and fieldwork.

I also would like to thank my colleague and friend **Dr. Salma Jallouli** for her guidance, advices and encouragement. I thank her for her help and sympathy.

My special thanks go to **Prof. Chahine Karmous** and **Dr. Ines Zouari** for their support, scientific advices and revisions in the hardest time of my thesis.

I address my sincere thanks to all my colleagues and friends, **Mustafa, Houcem, Nawel, Sihem, Maissa, Zeineb K., Zeineb H., Sana., Latifa, Marwa, Mokhtar.**

My deepest thanks to all **Sergio Esposito's team**, **Simone, Giorgia C., Valeria, Giorgia S. Gioia** and **Eloy** for their sympathy and wonderful reception. Thank you for all the warm moments and knowledge that we shared together.

I would like to thank the staff of IRA and CFPA Medenine, especially **Mr. Youssef Bohli** for the services provided to facilitate my work.

ملخص

تعتبر ملوحة التربة ومياه الري أحد معوقات الإنتاج الزراعي خاصة في المناطق القاحلة. إن الإفراط في استخدام المياه الجوفية، وتسرب مياه البحر ، وتوسيع استخدام الأراضي للزراعة في المناطق المالحة ، وتغير المناخ هي بعض الأسباب التي تجبر المزارعين على استخدام المياه قليلة المالحة لإنتاج المحاصيل ، مما يؤدي إلى زيادة التملح الثانوي في حلقة مفرغة. يُعرف استخدام المحاصيل والأصناف التي تتحمل الملوحة ، والإدارة السليمة للري ، بأنها الحل الأكثر استدامة لإنتاج المحاصيل تحت الملوحة. إلى جانب ذلك، من أجل زيادة الدخل إلى الحد الأقصى ، يلزم التسميد العقلاني. يعتبر النيتروجين (N) أحد العناصر الغذائية الأساسية اللازمة لتطوير النبات وإنتاجيته. يعد تطوير ممارسات فعالة لتحسين كفاءة استخدام النيتروجين تحت الملوحة أمرًا ضروريًا ويمكن أن يكون وسيلة للتخفيف من المشكلات الزراعية والبيئية المرتبطة بالإنتاج الزراعي تحت قيود الملوحة.

يهدف هذا العمل إلى دراسة التفاعل بين الملوحة والتمثيل الغذائي للنيتروجين في أربعة أصناف من الشعير تتناقض مع استجابتها للملوحة ("B1/100" و "Souihli" مقاومان للملوحة ; "Barley Medenine" و "ICARDA20" حساسان للملوحة). تمت زراعة أصناف الشعير لمدة 3 سنوات في الحقل تحت 8 مجموعات من المعالجات (أربع جرعات من التسميد بالنيتروجين:0 /50/100/ و 150 كجم نيتروجين/هكتار،ومستويان لملوحة المياه: 1.8 و 9.2 ديسي سيمنز/م) وأيضًا تحت ظروف خاضعة للرقابة (الزراعة في أصص و الزراعة المائية).

الهدف من هذا العمل هو تقييم الأداء الزراعي والفسيولوجي والكيميائي الحيوي للأنماط الجينية المختلفة ودراسة التباين الوراثي لكفاءة استخدام النيتروجين وتوزيع النيتروجين في أجزاء مختلفة من النبات تحت ضغط ملحي. كما يهدف أيضًا إلى تحديد السمات التي ينطوي عليها التحكم في كفاءة استخدام النيتروجين والمؤشرات الفسيولوجية والكيميائية الحيوية لتحمل النبات للملوحة.

أظهرت النتائج أن الأصناف المقاومة والحساسة لها آليات مختلفة في التمثيل الغذائي وإزالة السموم تحت ضغط الملح. تؤدي الملوحة إلى اضطراب امتصاص النيتروجين وتؤثر سلبًا على إنتاجية وجودة الحبوب ، و في المقابل تم تحسينها من خلال الإمداد بالنيتروجين. تم تحسين إنتاجية الحبوب بزيادة إمداد النيتروجين إلى 100 كجم نيتروجين/ هكتار، ولكن فوق هذه الجرعة تأثر المحصول سلبًا. أدى تراكم النيتروجين فالحبوب بشكل أساسي إلى تحسين عدد الحبوب بدلاً من تركيز البروتين للحبوب في الأصناف الوراثية الحساسة، بينما تم تحسين عدد الحبوب وكذلك محتوى البروتين في التراكيب الجينية المتسامحة.

تحت 150 كجم نيتروجين/هكتار ، يبقى النتروجين الزائد في القش في "ICARDA20" بينما يبقى النتروجين الزائد في الحواف في "B1/100" ؛ بينما أظهر "Souihli" تراكم النيتروجين الزائد وحوّله إلى بروتين في الحبوب. يمثل تراكم النتروجين في الحبوب 60٪ من إجمالي تراكم النيتروجين، بينما يمثل القش والحواف 30٪ و 10٪ على التوالي. قلل الري المالح من كفاءة امتصاص النيتروجين بنسبة 40.3٪ وكفاءة استخدامه بنسبة 28.5٪ ، ولكن زاد من كفاءة النقل بنسبة 18.6٪. أظهر التركيب الوراثي لـSouihli" "أعلى كفاءة امتصاص (65.3٪)، بينما كان "ICARDA20" الأكثر كفاءة في نقل النتروجين (45.75 كجم من الحبوب / كجم من النتروجين الممتص)، سجل هذان الصنفان الجينيان أعلى كفاءة لاستخدام النتروجين (25.5 كجم / كجم نتروجين).

أكد العمل في ظل ظروف خاضعة للرقابة أن إمداد النيتروجين يمكن أن يخفف من الآثار الضارة للملوحة ويحسن نمو النبات ؛ ويدعم الفرضية القائلة بأن أنشطة إنزيمية ، كفاءة استخدام النتروجين (NUE) ، تركيز الصوديوم وامتصاص البوتاسيوم قد تكون مؤشرات مفيدة تدل على تحمل الشعير للملوحة. تحت الملوحة ، حافظ "B1/100" على امتصاص النتروجين عن طريق تحفيز دورة GS/GOGAT تحت محتوى عالي من النيتروجين ، وتحفيز مسار GDH تحت معالجة منخفضة النيتروجين ، في حين أن "Barley Medenine" قلل من دورة GS/GOGAT وزاد نشاط GDH. ظلت HSP70 و PEPC دون تغيير في "B1/100" تحت الملوحة بينما انخفضت في"Barley Medenine". أدى التعرض للملوحة إلى زيادة نشاط APX و PEPC بسرعة في "B1/100" ، بينما تأخر في "Barley Medenine". زادت الملوحة من مستويات Cyt-G6PDH في "B1/100" ، بينما أظهر "Barley Medenine" انخفاضًا في G6PDH.

Resumé

La salinité des sols et de l'eau d'irrigation est l'une des contraintes à la production agricole, en particulier dans les zones arides. La surutilisation des eaux souterraines, l'intrusion d'eau de mer, l'extension de l'utilisation des terres pour l'agriculture aux zones salines et le changement climatique sont quelques-unes des causes qui forcent les agriculteurs à utiliser l'eau saumâtre pour la production agricole, augmentant, dans un cercle vicieux, la salinisation secondaire. L'utilisation de cultures et de variétés tolérantes au sel, une bonne gestion de l'irrigation sont connues comme la solution la plus durable pour la production végétale sous contrainte saline. En outre, pour maximiser les revenus, une fertilisation rationnelle est nécessaire. L'azote (N) est considéré comme l'un des macronutriments essentiels nécessaires au développement et à la productivité des plantes. Développer des pratiques de gestion efficaces de l'azote et améliorer l'efficacité de l'utilisation de l'azote sous salinité sont impératifs et peut être un moyen d'atténuer les problèmes agronomiques et environnementaux liés à la production agricole sous contrainte saline.

Dans ce contexte, ce travail vise à étudier l'interaction entre la salinité et le métabolisme azoté dans quatre génotypes d'orge contrastés vis-à-vis leur réponse à la salinité ("100/1B" et "Souihli" sont tolérants, "Barley Medenine" et "ICARDA20" sont sensibles). Les génotypes ont été cultivés durant 3 ans en plein champ sous 8 combinaisons de traitements (quatre dose de fertilisation azoté : 0,50,100 et 150 kgN/ha, et deux niveau de salinité d'eau: 1,8 et 9,2 dS/ m) et également sous des conditions contrôlées (sous serre et en hydroponie).

Le but de ce travail est d'évaluer les performances agronomiques, physiologiques et biochimiques de différents génotypes et d'étudier la variabilité génotypique de l'efficacité d'utilisation de l'azote et la distribution de l'azote dans différentes parties de la plante sous la contrainte saline. On vise également l'identification des traits impliqués dans le contrôle de l'efficacité d'utilisation de l'azote et les indices physiologique et biochimiques de la tolérance des plantes à la salinité.

Les résultats ont montré que les génotypes tolérants et sensibles ont des différents mécanismes de métabolisme et de détoxification sous stress salin. La salinité perturbe l'absorption de N et affecte négativement la productivité et la qualité du grain, qui ont été améliorées par l'apport azoté. La productivité en grain a été améliorée avec l'augmentation de l'apport d'azote à 100 KgN/ha, mais au-delà de cette dose le rendement a été affecté négativement. L'attribution de N

aux puits a amélioré principalement le nombre de grains plutôt que la concentration en protéines des grains dans les génotypes sensibles, tandis que le nombre de grains et aussi la teneur en protéines ont été amélioré chez les génotypes tolérants.

Sous 150 KgN/ha, l'excès de N reste dans la paille chez "ICARDA20" alors qu'il était attribué aux arêtes chez "100/1B"; tandis que "Souihli" a montré une accumulation de luxe de N excessif et le convertit en protéine de grain. Le génotype sensible "Barley Medenine" marqué par sa faible productivité a montré une altération de l'allocation de N à l'épi. L'accumulation de N dans les grains représente le pool majeur et représente 60% à l'accumulation totale de N, alors que la paille et les arêtes ont représenté 30% et 10% respectivement. L'irrigation saline a réduit l'efficacité d'absorption de N de 40,3% et l'efficacité de son utilisation de 28,5%, mais elle a augmenté l'efficacité de translocation de 18,6%. Le génotype tolérant "Souihli" a montré l'efficacité d'absorption la plus élevé (65,3%), tandis que le génotype amélioré "ICARDA20" a été le plus efficace dans la translocation de N (45,75 kg de grains/kg de N absorbé), ces deux génotypes ont enregistré l'efficacité d'utilisation de N la plus importante (25,5 Kg/KgN).

Le travail sous conditions contrôlées a confirmé que l'apport azoté peut atténuer les effets néfastes de la salinité et améliorer la croissance des plantes ; et supporte l'hypothèse que les activités enzymatiques spécifiques et leur occurrence, l'efficacité d'utilisation d'azote, la concentration de sodium (Na^+) et l'absorption de potassium (K^+) peuvent être des indices utiles de la tolérance de l'orge à la salinité. Sous salinité, "100/1B" a maintenu l'assimilation de N en stimulant le cycle glutamine synthétase/glutamate synthase (GS/GOGAT) sous une teneur en N élevé, et la stimulation de la voie glutamate deshydrogénase (GDH) sous un traitement à faible teneur en N, tandis que le génotype sensible "Barley Medenine" a réduit le cycle GS/GOGAT et a augmenté l'activité GDH. Les protéines de choc thermique70 (HSP70) et phosphoénolpyruvate carboxylase **(**PEPC) sont restées inchangées chez "100/1B" sous salinité alors qu'elles ont diminué chez "Barley Medenine". L'exposition à la salinité a rapidement augmenté les activités ascorbate peroxidase (APX) et PEPC chez "100/1B", alors qu'elle a été retardée chez "Barley Medenine". La salinité a augmenté les niveaux de glucose-6-phosphate déshydrogénase cytosolique (cyt-G6PDH) chez "100/1B", tandis que "Barley Medenine" a montré une diminution des isoformes de G6PDH.

Abstract

Soil and irrigation water salinity are one of the constraints to agriculture production particularly in arid areas. Underground water overuse, sea water intrusion, extension of land use for agriculture to saline areas and climate change are some of the causes that are forcing farmers to use brackish water for crop production, increasing, in a vicious circle, secondary salinization. The use of salt tolerant crops and varieties, a proper irrigation management are known as the most sustainable solution for crop production under saline constraint. Besides, an in order to maximize income, rational fertilization is required. Nitrogen (N) is considered as one of the essential macronutrients required for plant development and productivity. Developing effective N management practices and improving N use efficiency under salinity are imperative and can be a way to alleviate the agronomic and environmental problems linked to agriculture production under saline constraint.

In this context, this work aims to study the interaction between salinity and N metabolism in four barley genotypes contrasting for their response to salinity ("100/1B" and "Souihli" are tolerant, whereas "Barley Medenine" and "ICARDA20" are susceptible). Genotypes were cultivated during 3 years in field at the arid region EL Fjé-Medenine under eight treatments (four N rate which: 0, 50,100 and 150 kg N/ha, and two salinity level: 1.8 and 9.2 dS/m) and under controlled conditions (green house and hydroponic assay). The goal of this work is to assess the agronomic, physiologic and biochemical performances of genotypes grown under salinity and investigate genotypic variability for nitrogen use efficiency (NUE) and N partitioning pattern in different plant part. Additional goals are to identify morphological and physiological traits that may be involved in the control of NUE and identify physiological and biochemical index of plant tolerance to salinity.

The results showed that tolerant and susceptible genotypes have different N metabolism and detoxification mechanisms under abiotic stress. Salinity disturb N uptake, and negatively affected plant productivity and grain quality, which were enhanced by N supply. Agronomic performances was enhanced when N supply increased to 100 KgN/ha, however more addition of N caused a negative effect in plant productivity. Results showed that the distribution pattern of total N was different between genotypes: the improved genotype "ICARDA20" showed their benefit to enhance grain quality from low N supply. Under 150N excessive N remain in straw in "ICARDA20" while it was allocated to awns in "100/1B"; whereas "Souihli" showed a luxury accumulation of excessive N and convert it into grain protein. The sensitive "Barley

Medenine" marked by its low productivity showed an alteration in N allocation to the spike. Grain N content represent the major N pool and contributed by 60% to the total N uptake, while straw and chaff represented 30% and 10% respectively. Saline irrigation decreased the efficiency to uptake N by 40.3% and NUE by 28.5%, but increased N utilization efficiency (NUtE) by 18.6%. The tolerant "Souihli" showed the highest N uptake efficiency (NUpE) (65.3%) while the improved "ICARDA20" was the most efficient in using N (45.75 kg grain/kg uptaken N) which resulted in higher NUE for these two genotypes (25.5 Kg/KgN).

Assay under controlled conditions showed that N supply could alleviate the adverse effects of salinity and improve plant growth, and support the hypothesis that specific enzymatic activities and occurrence, NUE, sodium (Na^+) concentration, and potassium (K^+) uptake can be a useful index of barley tolerance against salinity. Under salinity, the tolerant "100/1B" was shown to support N assimilation by enhancing Glutamine Synthetase/Glutamate Synthase (GS/GOGAT) cycle under high N, and the stimulation of glutamate deshydrogenase (GDH) pathway under low N treatment, while the sensitive "Barley Medenine" reduced the GS/GOGAT cycle, and increased GDH activity. Heat Shock Proteins 70 and phosphoenolpyruvate carboxylase (PEPC) remained unchanged in "100/1B" under salinity while they decreased in "Barley Medenine". Exposure to salinity rapidly increased ascorbate peroxidase (APX) and (PEPC) activities in "100/1B", while it was delayed in "Barley Medenine". Salinity increased cytosolic glucose-6-phosphate deshydrogenase (cyt-G6PDH) levels in "100/1B", while "Barley Medenine" showed a decrease in G6PDH isoforms.

Abbreviations

APX: ascorbate peroxidase

BY: biomass yield

Cl^-: chloride

DWAP: dry weight aerial part

DWRP: dry weight root part

FWAP: fresh weight aerial part

FWRP: fresh weight root part

G: genotype

G6PDH: Glucose 6-phosphate dehydrogenase

GDH: Glutamate dehydrogenase

Gln: Glutamine

Glu : Glutamate

GOGAT: Glutamate synthase

GPC ; grain protein content

GS: Glutamine synthetase

GY: grain yield

HSP 70: Heat Shock Proteins 70

K^+: Potassium

MENA: Middle East and North Africa

N : nitrogen

Na^+ : sodium

NaCl: sodium chloride

Nb: number

NH_4^-: Ammonium

N**HI**: Nitrogen harvest index

NO_3^-: nitrate

NUE: itrogen use efficiency

NupE; nitrogen uptake efficiency

NutE; nitrogen utilization efficiency

PEPC: phosphoenolpyruvate carboxylase (PEPC

ROS: reactive oxygen species

S: salinity

WOS: weeks old seedlings

Y: year

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General Introduction

Increasing human population, climate change, water shortage and land degradation are real threats to food security. Subsequent urbanization and population growth are the two major menaces to the healthy and productive soils; in fact, population was around 0.6 billion in year 1700, 1.6 billion in 1900 and around 7.79 billion in 2020, the population is expected to reach 9.6 billion in 2050 and around 10.9 billion in 2100 (Zaman et *al.*, 2016). The variety of climatic and field conditions, the used germplasm, and the local expertise lead to the non-uniformity of crop production and the imbalance of global food production. In fact, 183 nations imports food from the countries when low populations performing intensive agriculture (Almousa, 2017).

In the past, the need for more food has been fulfilled by expanding the farmed area. Today, rising crop production is restricted by the suitability of land for agriculture and the limitation of water resources. In fact, more than one billion hectares of lands are affected by salinity (Saade et *al*., 2016).

Since centuries, salinity attracted most attention and considered as a worldwide problem due to its detrimental effect on fertile soil, crop productivity, agriculture sustainability and food security. Salinity occurs mainly in arid and semi-arid areas where the amount of rainfall is insufficient to compensate crop water requirement and leach soluble salts in the root zone. The soil salinity globally extending through more than 100 countries and more than 20% of the worldwide irrigated area is affected (Zaman et *al.*, 2016). In Tunisia, about 1.5 million hectares are considered as saline (Hachicha, 2007) and the water resources intended for irrigation are often of marginal quality. In Tunisia, 50% of the water resources represent a dry residue greater than 1.5g/l, and 23% more than 3g/l (Ben Mechlia, 2004). Salinity if not understood and managed timely can decrease production yield or totally eliminate the crop at certain high salinity level. Thus, the optimization of saline soil management, the efficient management of nutrients and water, and the introduction of salt tolerant crops, are keys to sustainable agriculture in saline soils.

Barley is widely cultivated in arid and semi-arid climate, characterized by its complexity and severity, and it is considered as elite crop to comply with this challenge. Barley crop often experiences multiple challenges arising from environmental stresses, such as salinity, drought, high temperature. In Tunisia, barley is the second cultivated cereal and is mainly used for animal feed and human food. Whereas its production does not meet the ever increasing needs. An external Nutrient input especially Nitrogen can improve barley yield production, but a high N input with a low N efficiency eventually results in environmental pollution and soil degradation. Therefore, improving crop production in saline environment through the improving of NUE is required among the major global targets. Improving NUE is a real challenge for successful

agriculture, so that yields can be increased with reduced N inputs. This challenge can be achieved through the improving of N fertilizer recovery and/or internal use of N by the plant. Breeding to develop barley genotypes, which can use N more efficiently would lower crop N fertilizer requirements without compromising yields and develop environmentally-friendly agricultural systems.

The works on the relation between salinity and nitrogen metabolism are limited, and the plasticity of nitrogen metabolism under salinity are not well studied, hence the need to conduct such work. In this context, the present work deals with a deep understanding of the interaction between salinity and nitrogen metabolism in different barley genotypes cultivated at the arid region EL Fjé-Medenine. A proper understanding of the mechanisms of genotypic adaptation to salinity will therefore help researchers and scientists to make more effective crop management program.

Research objectives and thesis plan

Barley (*Hordeum vulgare.* L) was ranked the second most produced cereal in Tunisia. It is generally cultivated in the semi arid and arid climate. Barley productivity is generally low and the national average of yields hardly exceed 7.5 qx / ha. Therefore, improving crop yields and production that can be achieved by additional irrigations is required among the major targets in Tunisia. However, the main constraint is the high salinity of the irrigation water in these cultivation areas. The cultivation of tolerant barley genotypes can be a way of mitigating this constraint. Furthermore, an adequate nitrogen management in saline environment can be a key to enhance barley production. Nitrogen Use Efficiency (NUE) is considered from three of view: agronomy (grain yield per unit of N supply), environment (possible contamination), economics (maximization of farmers' income) (Barrachlough et *al*., 2010). Therefore, enhancing barley productivity and quality in arid regions, minimize production cost and environmental pollution through the use of genotypes with higher NUE is a real challenge. To meet this challenge and well understand the interaction between saline irrigation and nitrogen fertilization, four barley genotypes was cultivated under field conditions in arid region, and assessed under four Nitrogen fertilization level (0,50,100,150 kgN/ha) and two saline irrigation level (1.8 and 9,2 dS/m) from 2016 to 2019 in Medenine **(chapter I and II).** Tolerance to salinity is very complex and involves several mechanisms (Roy et *al*., 2014). Whereas, field trials are difficult to establish some physiological and biochemical data which can be subject to strong environmental effect. Therefore, a morphological, physiological and biochemical studies under controlled conditions were conducted to improve our understanding on tolerance mechanisms to salinity, the regulation of N metabolism under salinity and how N fertilizer could alleviate the detrimental effects of salinity **(Chapter III and IV).**

The thesis plan is as follows:

Literature review: This thesis starts with a literature review regarding the salt tolerance strategies in plants and the agronomical and physiological bases of Nitrogen use efficiency under salinity, with an emphasis on the importance of barley production in arid region.

Chapter I: entitled "Yield and N uptake distribution pattern in contrasting barley genotypes (*Hordeum vulgare* L.) grown in Mediterranean arid environment" reports the analysis of the agronomic performances of the four barley genotypes grown under the different field conditions. The genotypic differences for N uptake and its partitioning in different plant parts was investigated and the optimal nitrogen supply in such environment were performed.

Chapter II: entitled "NUE components in contrasting barley genotypes (*Hordeum vulgare* L.) grown in Mediterranean arid environment", reports the evaluation of Nitrogen use efficiency and the regulation of N uptake and utilization efficiency in the four genotypes grown under different field conditions.

Chapter III: entitled "Interactive effects of nitrogen nutrition and salinity on physiological responses in barley (Hordeum vulgare L.)" aims to understand of the physiological mechanisms allowing N supply to alleviate the adverse effects of salinity and improve plant growth and productivity in the four contrasting barley genotypes grown under controlled conditions. This chapter deals with the hypothesis that Nitrogen use efficiency (NUE), sodium and potassium accumulation could be a useful index of barley tolerance against salinity.

Chapter IV: entitled "Salt Stress Induces Differentiated Nitrogen Uptake and Antioxidant Responses in Two Contrasting Barley Landraces from MENA Region"; an hydroponic culture was performed in climate room under controlled conditions, where two genotypes (the most stable genotype under salinity and the most sensitive genotype) were grown under different N and salinity levels. This chapter aimed to investigate the effects of the interaction between salinity and N metabolism in two contrasting barley landraces and understand how these landraces respond to the detrimental effects induced by salt stress and how salinity regulates the N metabolism. Therefore, specific enzymatic activities and protein occurrence involved in responses to salinity and nitrogen assimilation were analyzed in order to identify stress responsive sensors and the distinct regulations in the main metabolic pathways in order to adapt to environmental conditions was investigated.

Literature review

I. The importance of barley in arid area

Barley was ranked the fourth most produced cereal in the world (FAOSTAT, 2017). It represents one of the first grown grains in the Near East, and domesticated around 10,000 years (Badr et *al.*, 2000). Migration of people with their seeds led to a substantial diversification and adaptation to new regions. Actually barley crop is found worldwide. Barley is among the most tolerant cereals against abiotic stresses (Shen et *al*., 2016). Barley is widely cultivated to be used for human food, animal feed and malts especially in arid and semi-arid areas characterized by harsh environment subjected to several stress conditions such as drought, salinity, heat, water scarcity and nutrient deficiency (Lee et *al*., 2020). In Tunisia, barley is the second most produced cereal; its production between 2008 and 2018 was around 481.57 thousand tons on average (Onagri, 2019). Barley represents a main resource that supports small farmers and often replaces wheat and other cereals in arid environment (Hammami et *al*., 2017). In the Tunisian arid areas, barley is the most produced cereal, and "Medenine" is the arid region the most productive of Barley (Figure 1), where barley production ranged from 8100 to 28600 tons across the years (2007-2017) whereas wheat production do not exceed a maximum of 860 Tons (onagri, 2019). In addition, barley is known as plant model organism used to study genetic resistance to abiotic or biotic stress and develop plant breeding methodologies, since it can tolerate a wide range of single or combined environmental stress (Gürel et *al*., 2016).

Therefore, the genome of barley is a reservoir of multiple stress response alleles representing a precious item for genetic engineering in some other crop species. The haploid genome of barley has a size of about 5.3 Gbp. Currently, it is among the largest diploid genomes sequenced and contains 83,105 putative genetic loci comprising 39,734 ones with high confidence (Lee et *al*., 2020). Therefore, farmers throughout years have selected a number of desired genotypes generally described as landraces. Farmers' selection, together with natural selection favored the diversity and generated a rich gene pool of variation found now in local varieties which formed the elemental material for modem plant breeding (Bothmer et *al*., 2003).

Cultivated barley (*Hordeum vulgare* L.) is a monocotyledonous, autogamous cereal. It is a diploid species with 7 pairs of chromosomes (2n=14). *Hordeum vulgare* L. is one of 32 species of the genus Hordeum, originating from the wild forms. All of these species belong to the kingdom of Plantae, to the sub-kingdom of Tracheobionta, to the superdivision of Spermatophyta, to the division of Magnoliophyta, to the class of Liliopsida, to the subclass of Commelinidae, to the order of Cyperales, the family of Poaceae and the genus of Hordeum (Bothmer et *al*., 1995).

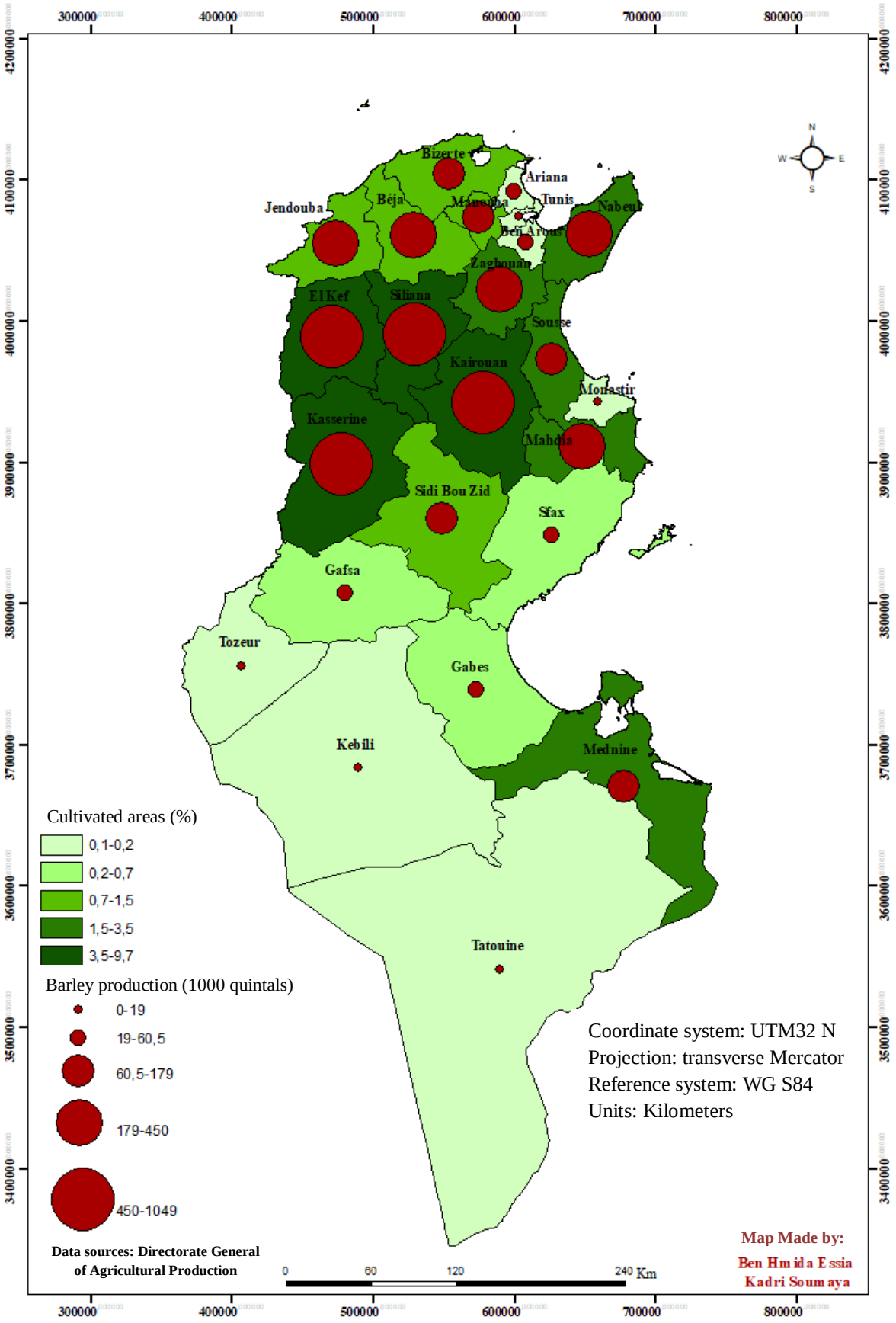

Figure 1: Distribution of average barley production between 2007 and 2017 (Source: Onagri, 2019)

Barley is classified based on head morphology into two types: two and six rows barley. Six-row barley characterized by fertile middle and lateral spikelets (*Hordeum hexastichum* L. (escourgeon). In the two-row barley, only the middle spikelets are fertile (*Hordeum distichum* L.) (Souilah, 2009). The existence of intermediate 4-row barley (*Hordeum vulgare ssp. Tetrastichum*) has also been reported by Harlen and Hayes (1920). This type is the result of a cross between the two and the six row barley. Soltner (2005) classifies barley according to the need for growing cold in 3 groups: winter barley, spring barley and alternative barley.

II. Salinization and salt stress tolerance strategies in plants

Salinization is the process of enriching soil with soluble salts resulting in the formation of saline soil (Mermoud, 2006). It can be natural or induced by agricultural activity in particular irrigation (with saline water) and the excessive use of mineral fertilizers. A soil is generally classified as saline when its electrical conductivity exceeds 4 dS/m, which is the equivalent of about 40 mM NaCl for most plants (USDA-ARS, 2008). The accumulation of salts in the soil, particularly in the rhizosphere, affects its fertility and agronomic aptitudes. Salinity is exacerbated with climate change especially the increase in global temperatures and the scarcity of precipitation, which leads to the loss of arable land. It is estimated that about 2,000 hectares of irrigated land in arid and semi-arid zones across 75 countries are damaged by salinity every day, costing around US $ 27.3 billion per year, due to the crop production lost (Qadir et *al*., 2014). In 1990, the annual cost of loss caused by salt land degradation was US $ 264 per hectare, which elevated to US $ 441 per hectare in 2013 (Zaman et *al*., 2016*).* The problem of soil salinization seems to be more accentuated in the Mediterranean region, more particularly in arid and semi-arid climates, where evaporation exceeds precipitation.

1. Effect of salinity on plants

Soil salinity induces various metabolic changes in plants, such as the reduction of water absorption, ion toxicity, the alteration of nutrient uptake and metabolism, the reduction in chlorophyll content and photosynthesis, the accumulation of reactive oxygens pecies (ROS), and all such changes subsequently decreased plant growth and productivity (Acosta-Motos et *al*., 2017). Generally, salinity subjects plants to four types of constraints: osmotic, ionic, nutritional and oxidative.

1.1. Salinity components

1.1.1. Osmotic stress

The presence of salt in the soil solution threatens the plant's water supply by decreasing the water potential around the roots. In fact, more the soil solution contains salts, more the osmotic pressure is higher, and more it is difficult for the roots to extract water from the soil. This generates a physiological dryness caused by the loss of turgor due to excessive water outflow from cells and the increase in the concentration of solutes in the intracellular compartments (Song et *al*., 2005). The osmotic potential becomes more negative and the cellular water potential becomes greater than that of the extracellular environment and that of the soil, which makes it impossible to move water from the soil to the leaves. Hence the perturbation in plant growth. So it is vital that plant adjust its osmotic potential in order that cellular water potential remains lower than that of the soil. This phenomenon ensures, on the one hand, the continued absorption of water from the soil; and on the other hand, the retention of intracellular water and maintenance of turgor (Ottow et *al*., 2005).

1.1.1. Ionic stress

During salt stress, plants absorb high concentrations of NaCl despite other ions essential for its growth. Indeed, the Na^+ ions compete with K^+, $Ca^{2+,}$ Mg^{2+} and Mn^{2+} while the Cl^- ions limit the absorption of the $NO_3^{-,}$ PO_4^{2-} and SO_4^{2-} ions (Safdar et *al.*, 2019). The accumulation of sodium Na^+ and chloride Cl^- ions to toxic levels induces ionic toxicity in tissues which cause cell dysfunction (Chinnusamy et *al*., 2004). High cytosolic concentrations of Na^+ or Cl^- are not compatible with many metabolic processes (Roy et *al.*, 2014). The specific effects of these ions are numerous and affect leaf area, stomatal conductance, chlorophyll content, membrane integrity, enzymatic activities, nuclear function, absorption of nutrients as well as the functioning of the photosynthetic apparatus (Najar et *al*., 2019).

1.1.2. Nutritionnel stress

Nutritional stress is mainly the consequence of the antagonism between Na^+ ions and essential cations on the one hand, and Cl- ions and anions on the other hand (Haouela et *al*., 2007). Disturbances in the absorption and transport of certain essential ions such as K^+, Ca^{2+}, Mg^{2+}, NO^{3-} under salinity have been reported in several studies. Sodium inhibits the absorption of elements, either directly through competition for the same type of transporters, or indirectly, by inhibiting root growth, or even through its deleterious effect on soil structure (Ashraf et *al.*, 2018). In the same context, it has been observed that a high concentration of Cl^- reduces the absorption of NO_3^- and phosphate (Singh et *al*., 2016).

1.1.3. Oxydative stress

Under salinity, the electron transport chain is negatively affected and the absorption of atmospheric carbon dioxide (CO_2) is reduced, causing a greater stomatal closure and a lower NADPH utilization via the Calvin cycle (Suo et *al.*, 2017) which favor the electron acceptor behavior of molecular oxygen and the accumulation of ROS (Figure 2) . Oxidative stress is due to the rapid and massive production ROS which can be free radicals like superoxide (O_2^-) and hydroxyl (OH^-) or active forms of oxygen such as hydrogen peroxide (H_2O_2) and (ONOOH)) (Lisar et *al.*, 2012). These radicals, produced as a result of the metabolism alteration of the chloroplasts and mitochondria during salt stress, and induce cellular and membrane changes that can lead to cell death (Parida and Das, 2005). These compounds induce the oxidation of different cellular components (such as membrane lipids, proteins, nucleic acids), inhibit photosynthesis (by promoting lipid peroxidation) and can even cause denaturation of proteins and mutations in DNA (Hernandez et *al.*, 2001). They also damage the structure of membranes and cause the degradation of chlorophyll, which in many cases leads to the appearance of necrosis on plant leaves (Turki, 2011).

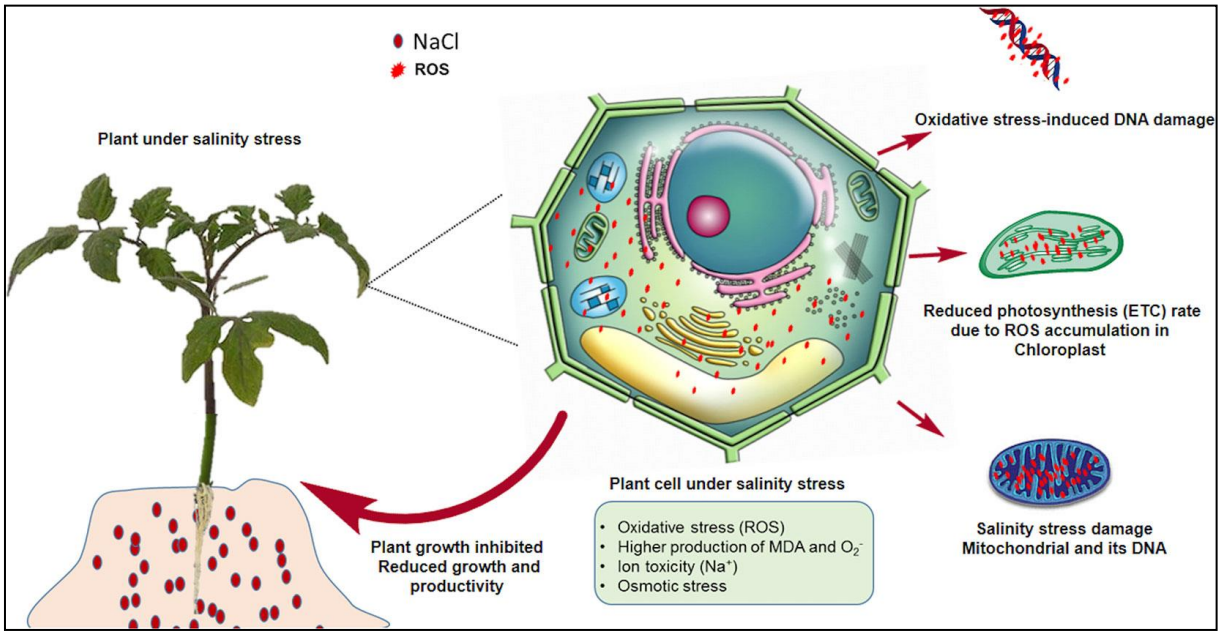

Figure 2: Accumulation of ROS in plants under saline conditions. Toxic levels of ROS restrict plant growth by inhibiting the electron transport chain, and photosynthesis rate, and by damaging mitochondria and DNA (Khan et *al.*, 2019).

1.2. Salinity effect on germination

Several studies have shown that the high concentration of sodium alters seed germination, delays its initiation, and increases the risk of seed damage (Karmous, 2007). In fact, due to

osmotic stress, it is very difficult that the embryo absorbs the necessary quantity of water to trigger the metabolic processes of germination (Slama et *al*., 2005). Some authors have explained the inhibition of germination by the variation in the hormonal balance in the seed due to the high salinity (Debez et *al*., 2001).

1.3. Salinity effect on growth and development

The limitation of vegetative growth under salinity has been demonstrated in different plant species. The action of salt stress on plant development varies according to many factors like the intensity and duration of stress, type of salt, local environmental conditions (air humidity, quantity of water in the soil, etc.), genus, species, variety and stage of development (Ashraf et *al*., 2018). Salt negatively affects the growth process through several physiological modifications such as ionic imbalance, water status, mineral nutrition, stomata behavior, photosynthetic efficiency, and carbon uptake (Shaheen and Hood-Nowotny, 2005).

Several studies have shown that biomass reduction is the major effect of salinity. According to Munns (2002), the exposure to high salinity exhibited a remarkable reduction in plant size, fresh and dry biomass as well as other vegetative development traits such as the emission of tillers in cereals.

The first damage observed under stressful conditions is the rapid inhibition of the emission of young leaves and the reduction of old leaves stomatal conductance. Also, a reduction in the initiation of new seminal or lateral roots has been observed (Cramer and Bowman, 1991).

In cereals, salinity dramatically decreases the number of tillers and spikelets and reduces the flowers fertility. These physiological disturbances damage some essential metabolic processes in plants and result in the decrease of crops production (Munns, 2002).

1.4. Salinity effect on photosynthesis

Water deficit caused by salinity causes the closure of stomata and the decrease of their conductance leading to reduce carbon uptake and assimilation (Parida et *al*., 2005). This effect can be observed rapidly,few hours after exposure to salt (Hernández et *al*., 2002). The decrease in the assimilation of intercellular CO_2 which is essential for RuBisCO (Ribulose-1,5-Bisphosphate Carboxylase), -a key enzyme in carbon metabolism- cause the decrease in photosynthetic activity (Jiang et *al*., 2006). Salt reduces the activity of other enzymes involved in photosynthesis such as Phosphoribulose Kinase and Sucrose Phosphate Synthase (Lisar et *al*., 2012). In addition, the reduction of leaf area which represents the photosynthetic seat decreases photosynthesis rate.

In the long term, salinity can affect photosynthesis due to salt ions accumulation in leaves and reduces chlorophyll and carotenoid concentrations (Duarte et *al*., 2013). The action of salt on plant photosynthesis is mainly due to the disruption of photosynthetic electron transport and/or the alteration of the Calvin Cycle enzymes (Acosta-Motos et *al*., 2017).

1.5. Salinity effect on biochemical traits

Salt inhibits the synthesis of the majority of existing proteins and induces the synthesis of new proteins called stress proteins, such as osmotin, dehydrins and aquaporins (Lisar et *al*., 2012).
Changes in cell composition are often proved during salt stress. Thus, Mansour et *al.* (1993) found that salt can alter certain cytoplasmic structures such as microtubules, microfibrils and ribosomes. The lipid constituents of cell membranes are also affected. Also, high salt concentration leads to an increase in the levels of phytohormones such as abscisic acid and cytokinins (Munns and Sharp, 1993). Abscisic acid plays a crucial role in regulating growth and controlling stomatal conductance. It decreases the levels of active gibberellins and consequently inhibits leaf elongation (Munns and Tester, 2008).
In order to study the impact of salinity on reproduction, a study about the accumulation of salt in the barley meristem carried out by Munns and Rawson (1999), results showed that salt stress, applied during organogenesis, caused abortion of the ovaries and irreversible damage to the fertility of the ear.

2. Salinity tolerance

Tolerance is the ability of plants to grow and complete their life cycle in an environment that is highly concentrated in soluble salts, especially NaCl.

2.1. Genetic variation for salt tolerance

Tolerance to a salt constraint is a complex trait that varies between plants; genetic control for salt tolerance is still poorly understood because of its complexity (Mrani Alaoui et *al*., 2013). This variability exists between families, species, and varieties and even between individuals of the same variety (Tester and Davenport, 2003). Based on their behavior with respect to salt stresses, plants can be classified into glycophytes which show a sensitivity to salt or halophytes which can adapt to growing in saline conditions. Halophytes are able to accumulate large amounts of sodium in their aerial parts up to 30% of their leaf dry matter (Tester and Davenport, 2003). Halophytes have been classified into two types: includer and excluder type. In includer plants, Na^+ and Cl^- ions enter plants through the roots and they are carried through the xylem to the stems and leaves where they will be stored. Thus, salt is trapped in the vacuoles thanks

to molecular pump systems, and it isolated from vital cellular constituents and kept away from the meristems of growing shoots and young leaves (Berthomieu et *al*., 2003 cited by Bouatrous, 2013). Whereas, in excluder plants Na^+ and Cl^- ions, transported by the xylem, are poorly retained and re-transported by the phloem to the roots of the plant. The passage of salt from the roots to the leaves is prevented by a barrier that exists in the endoderm (Munns and Tester, 2008).

2.2. Mechanisms of salinity tolerance in plants

Tolerance to salinity is strongly dependent on the efficiency of the plant in removing excess of salts and in restricting the entry of Cl^- and Na^+ ions. To cope with the harmful effects of salinity, plants develop various physiological and biochemical mechanisms that help plants to adapt to salinity. These mechanisms allow the regulation of ion imbalance, the reduction of stored salts, the maintenance of the internal osmotic balance as well as the activation of the antioxidant systems. According to Roy et *al.*, (2014), salinity tolerance strategies can be classified into three main categories which are: ionic selectivity, vacuolar sodium compartmentalisation and osmotic adjustment. The activation of antioxidant enzyme, the compatible solutes and osmo-protectants biosynthesis, antioxidant and polyamine synthesis, nitric oxide (NO) generation, and hormonal alterations are main tolerance mechanisms to salinity (Khan et *al*., 2019).

2.2.1. Ionic selectivity

Ion selectivity or exclusion of toxic ions consists of excluding sodium from the cytoplasm towards the outside of the cell. In this case, the plants limit the entry of saline elements and reject them in the apoplasmic compartment (Munns, 2005). Exclusion begins with the selectivity of the root membrane (Apse and Blumwald 2007). The transport processes of Na^+ and Cl^- mainly in the roots reduce the accumulation of Na^+ and Cl^- to toxic concentrations within the leaves. The changes due to salinity cause a deviation in metabolism and further energy requirement. Sodium exclusion is achieved by the combined action of a series of SOS ("salt overly sensitive") proteins (Khan et *al*., 2019). SOS1 is a Na^+/ H+ antiporter located in the plasma membrane, and plays a key role in this mechanism of sodium exclusion to the external environment (Numan et *al.*, 2018). Also, it has an essential role in regulation of the long-distance diffusion of Na^+ between roots and shoots (Abbas et *al*., 2017). In saline environment, the overexpression of SOS1 improves salt tolerance levels (Fan et *al*., 2019). SOS2 and SOS3 jointly regulate the SOS1 activity but also that of the vacuolar antiport NHX1 (Liu et *al*., 2000; Zhu, 2002; Qiu et *al.*, 2004) (Figure 3). On the other hand, in order to reduce sodium accumulation in the aerial part of plant, SOS protein complex interacts with the transporter

HKT1 (Rus et *al.*, 2001) located on the membrane plasma and responsible for the recirculation of sodium from the leaves to the roots via the phloem (Berthomieu et *al.*, 2003; Hauser and Horie 2010). The CCC transporters ("cation-chloride cotransporter"), responsible for the long-distance transport of chlorides, are also involved in the exclusion mechanism (Brumos et *al.*, 2009). Some physiological studies have shown a process of cytoplasmic sodium expulsion towards the apoplasm or vacuole in order to protect the aerial organs cytoplasm (Munns and Tester, 2008).

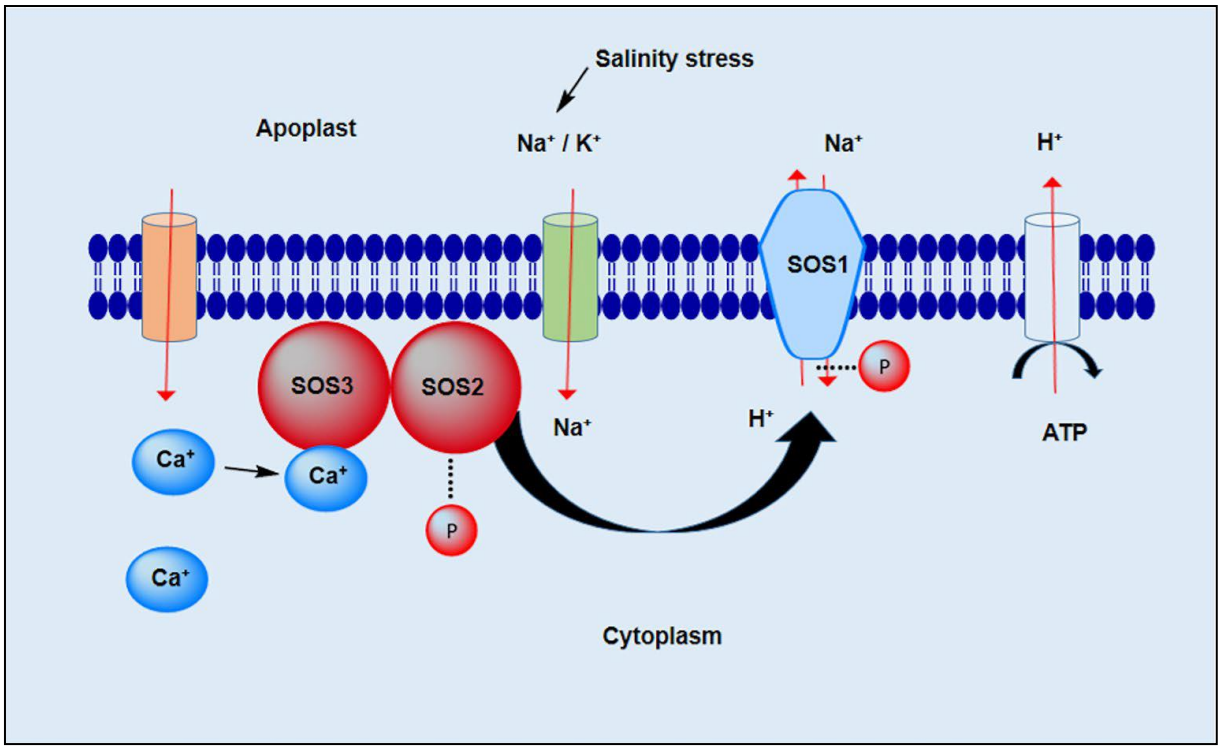

Figure 3: SOS pathway under salinity stress. The ions transport across the membrane is carried out by different carrier proteins like antiporters, symporters and channel proteins. Under salinity, the ion hemostasis in the cell is fundamental for its survival. (Khan et *al.*, 2019).

2.2.2. Vacuolar compartmentalization

The sequestration of Na^+ and Cl^- ions in vacuoles represents an adaptive mechanism to salt stress adopted by certain tolerant plants (Munns, 2002). It is reported that the efficiency of intracellular salt compartmentalization can explain the differences in salt tolerance between species (Bouchoukh, 2010). The compartmentalization of ions between organs (roots and aerial parts), tissues (epidermis and mesophyll) or between the cellular compartments (vacuole and cytoplasm) is among the most effective strategies to avoid the toxicity of Na^+ ions on the metabolic sites of the cytoplasm (Jabnoune, 2008). As vacuoles are closed compartments in the cell, salt is isolated from other vital cellular constituents (Munns, 2002). The vacuole is loaded

with sodium via the action of a sodium/proton (Na^+/H^+) antiport. The energy required for vacuolar compartmentalization mechanism is supplied by the proton pumps ATPases (H^+ -adenosine triphosphatases) and PPases (H^+ -pyrophosphatases) (Horie and Schroeder 2004).

2.2.3. Osmotic adjustment

The vacuolar accumulation of sodium causes a local decrease in the osmotic potential. The osmotic adjustment mechanism is to maintain the osmotic balance between the vacuole and the cytoplasm and to prevent the influx of water leaving the cytoplasm; for this reason plant synthesizes and accumulates solutes in the cytosol (Levigneron et *al.*, 1995). These osmoprotectors are uncharged, at neutral pH, hydrophilic and qualified as compatible because they do not disturb the interactions between the macromolecules and the solvent. Two main categories of osmoregulators can accumulate under stress: the first category groups together simple sugars (fructose and glucose), complex sugars (trehalose, raffinose and fructans) and polyols (mannitol, glycerol and inositols),whereas, the second category includes tertiary and quaternary derivatives of amino acids (proline, glycine-betaïne) as well as sulfonium compounds such as choline (Jabnoune, 2008). These compatible solutes can stabilize proteins and cellular structures and increase the osmotic pressure of the cell. They also help detoxify ROS and preserve the activity of enzymes in saline solutions. The accumulation of these compounds in the cytosol allows the increase in osmotic pressure and the establishment of a new equilibrium between the apoplastic solution and the vacuole (Levigneron et *al*., 1995).

2.2.4. Role of antioxidants in salt tolerance in plants

H_2O_2 and ROS accumulated under salinity are oxidizing compounds that can destruct the plasma membrane and endomembrane systems (Ahanger and Agarwal, 2017; Foyer, 2018). However they can act as signals of stress, thus activating the expression of a number of stress-responsive genes (Boubakri et *al.*, 2013), and inducing antioxidant enzyme activities and non-enzymatic compounds such as glutathione,ascorbic acid (Asc) and phenolic compounds (Khan et al., 2019). Antioxidant enzyme such as superoxide dismutase (SOD), gluthation peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) can adjust intercellular ROS to a stable state (Bose et al., 2014, Ahanger et al., 2018). Tuteja et *al.* (2013) indicated that the proteins DESD-box helicase and OsSUV3 dual helicase improve salinity tolerance by maintaining or enhancing photosynthesis and antioxidant enzyme. Recently, Ben Azaiez et al. (2020) proved that glucose 6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49) located in cytosol and plastids enhance salinity tolerance by providing NAPDH for scavenging enzymes.

2.2.5. Role of Nitric Oxide (NO) in Salt Tolerance in Plants

Nitric oxide (NO) is a volatile gaseous molecule essential for the preservation of different physiological and biochemical processes in plants cells, e.g seed germination, root growth, respiration, stomata closure, flowering, stress signaling, cell death, and stress responses (Besson-Bard et *al*., 2008; Zhao et *al*., 2009). Under stress, NO regulates various genes involved in enhancing tolerance to salinity, including different redox-related and antioxidant genes encoding for enzymes (such as GPX, GR, SOD, CAT, and APX), and represses lipid peroxidation or malondialdehyde (MDA), therefore restoring growth (Bajguz, 2014). In order to preserve high K^+/Na^+ ratio in cell cytoplasm of plants subjected to salinity, NO raises plasma membrane expression and/or tonoplast H^+-ATPase and H^+-PPase (Sung and Hong, 2010). Furthermore, NO allows the cell to accumulate different compatible solutes, such as organic osmolytes, proline, and soluble sugars, to promote cell turgor (Guo et *al*., 2005).

III. Nitrogen Use efficiency under salt stress

1. Nutrient availability in saline environment

Plants need mineral nutrients for growth and development. Nitrogen, phosphorus and potassium are the main nutrients required for plant growth. The availability of nutrient is a determining factor of crop productivity.

Nitrogen (N) is a major element for plant survival. It covers important portion of earth's surface; and represents a dominating element in the atmosphere in the form of gaseous (N_2) which is not immediately available to plants. Plant assimilate N in order to maintain vegetative growth and yield. In saline environment N deficiency represent a limiting factor for plants growth and productivity. The N deficiency changes leaf color to yellow and cause the abscission of older leaves and the remobilization of the remaining N to the younger leaves. Deficient N nutrition causes stem and tiller hardness (Taiz and Zeiger, 2010). Thus, good N management under salinity is essential practice to increase yield crop. The application of N fertilizer affects growth dilution to alleviate adverse effects salinity and improve economic crop yield (Murtaza et *al*., 2013). The requirement of N for crop grown under saline environment is different from that of crops grown under normal conditions because of the physical and chemical properties of saline soils. Salt-affected soils are generally characterized by high electrical conductivity (EC), pH and sodium adsorption ratio (SAR), calcareousness, poor physical soil conditions, low organic matter, low biological activity (Murtaza et *al*., 2013). Optimal supply of N fertilizer partially alleviate the detrimental effects of salinity on photosynthesis and photosynthesis-related traits,

yield and its components through mitigating the nutritional requirements of salt stressed plants (Sultana et *al*. 2001, Singh and Kashyap 2007), and also can increase salt tolerance of plants (Sairam and Tyagi, 2004).

2. Nitrogen Use Efficiency (NUE)

Because an important amount of the soil nitrogen is not used by the plant, Nitrogen Use Efficiency (NUE) is an indispensable parameter to measure the crops use of available or supplied N. NUE has been widely used to characterize the reactions of plants to different levels of available nitrogen; and it differs between plants cultivated for biomass production or grain yields. NUE is composed by two components: N uptake efficiency (NupE) and N utilization efficiency (NutE) of the amount of N already absorbed. The first component (NupE) defines the ability of the plant to absorb the soil N such as nitrate and ammonium ions; and the second component describes the ability to use the uptaken N from the soil to produce dry matter or grain yield (Cormier et *al*., 2016).

There is genetic variability in N uptake efficiency and N utilization efficiency for the majority of crop plants. In fact, NUE varies considerably from one species to another and from one genotype to another within the same species (Hirel et *al*., 2007). This genetic diversity of NUE has been demonstrated in several species such as durum wheat, rice and corn. Currently, it represents a selection tool used for the development of new cultivars with better uptake and utilization of N.

NUE is a multi-gene trait that can be influenced by several factors such as soil texture, climatic conditions, soil bacterial flora, sources of N existing in the soil (organic or inorganic) (Karrou, 2001). NUE can be improved by fertilization techniques (agronomic approach) and genetic improvement (genetic approach). The objective of the agronomic approach is to ensure good management of N fertilizers through a better adequacy between the supply and demand of crop. Thus, the importance to make available to the plant, at the appropriate times, the exact quantities of N allowing it to satisfy its requirements without losses. Certainly, these applications depend on the richness of the soil in N, the humidity of the soil, the stage of crop development and its production potential (varieties) (Garnett et *al*., 2005). In addition, studies aimed at improving the NUE are currently being carried out using conventional breeding and molecular genetics. All these studies, originally based on agronomic, physiological and genetic approaches, are currently supplemented by the use of high throughput techniques, making it possible to obtain gene expression profiles, protein and metabolite accumulation during plant development in its various organs and depending on the level of N fertilization (Hirel and Gaillais, 2013).

2.1. Salinity effect on N metabolism

Salinity affects considerably N metabolism from N uptake and NO_3^- reduction until NH_4^+ assimilation, which leads to a severe morphological, biochemical, physiological, and molecular changes. All of these changes reduce plant growth and crop production (Achraf et *al*., 2018). N is the most important nutrient that influence plant growth, it is a essential component of several compounds such as amino acids, amides and proteins, quaternary ammonium compounds, and polyamines; these compounds control several cellular activities and involved in different mechanisms of salt tolerance in plants (Zaki, 2016; Arghavani et *al*. 2017). Salinity depresses nutrient uptake and induces nutrient deficiency in plants, its effect on N metabolism is multifaceted and can differ depending on multiple factors such as plant species, the level and duration of the salt stress, the stage of plant growth, amount and form of supplied N in (Teh et *al*. 2016, Ashraf et *al.*, 2018). These changes influence noticeably plant N status and cause severe deleterious effects on plant growth.

Salinity disturbs N metabolism probably due to the reduction of N uptake, the inhibition of NO_3^- reduction and NH4+ assimilation, the alteration of activities of enzymes involved in N metabolism, the reduction of amino acid synthesis, the increase of hydrolyzing activity (Ashraf et *al.*, 2018).

2.1.1. Salinity effect on N uptake

Nitrate (NO_3^-) is the predominant and preferred source of N in plants and its absorption is faster than that of ammonium (Morot-Gaudry et *al.*, 2006). The absorption of these ions involves the crossing of biological barriers from root hairs to loading in the xylem (Meyer and Stitt, 2001). It requires the establishment of active transport systems to overcome the gradients of ionic concentrations between the different cell compartments and ensuring the selective passage of ions (Cerezo, 2001). Thus, plants have developed complex and highly regulated absorption systems to ensure their N supply. To ensure the absorption of inorganic N, there are two types of specific transporters: high and low affinity transport system (Glass et *al*., 2002). High affinity transport system (HATS) ensures the influx of nitrate but has a low capacity. This system is saturable beyond 200 µM. The particularity of this system is that it has two components: one constitutive (cHATS) and the other inducible (iHATS) (Morot-Gaudry et *al.*, 2006). Beyond 200-500 µM of external nitrate low affinity transport system (LATS) interpose. This system is non-saturable up to 50 mM nitrate (Morot-Gaudry et *al*., 2006).

Salinity can reduce N uptake via the antagonism between ions. In addition, the damage membrane structure of roots, the reduction of water absorption caused by osmotic changes in

root zone and the reduction of transpiration rate lead to reduce growth rate of plants, thus decrease plant N demand (da Silveira et *al.*, 1999; Ullrich, 2001; Gessler et *al.*, 2005; Debouba et *al.*, 2007).

2.1.1.1. Ionic antagonism

In saline environment, the process of N uptake is mostly inhibited due to the antagonistic effect of salt ions (Cl^- and Na^+) with nitrate and ammonium (NO_3^- and NH_4^+), and the disruption in loading of N ions into root xylem (Abd-El-Baki et *al*., 2000; Parida and Das 2004). The accumulation of N in the aerial part of plants can be reduced due to the salt anion Cl^- which is known to compete with NO_3^- (Abdelgadir et *al.*, 2005). Some authors studied the various sources of Cl^- and demonstrated that Cl^- from $CaCl_2$ but not KCl, can inhibit NO_3^- uptake. Only under higher concentration (100–200 mol/m^3) that Cl^- originated from KCl can inhibit NO_3^- uptake (Kafkafi et *al*., 1992). Also Na^+ has an antagonistic effect with NH_4^+ and could decrease significantly NH_4^+ uptake under saline conditions (Dluzniewskaet *al.*, 2007). The decrease of NH_4^+ uptake and the increase of Na^+ concentration in soil solution have been reported in different plants such as Sorghum bicolor L., Spartina alterniflora, Triticum aestivum L., Tageta patula, Gossypium hirsutum L. (Ashraf et *al*., 2018).

2.1.1.2. Plant water absorption

Salinity can impair N uptake through the reduction of plant water absorption caused by the changes in soil water potential. Many studies have demonstrated that due to salt ions, osmotic effects in soil solution reduce water absorption and the mass nutrient flow (including N) to the roots, which lead to a considerable N uptake reduction **(**Ehlting et *al.*, 2007; Zakery-Asl et *al.*; 2014).

2.1.1.3. Plant N demand

Salinity can restrict N uptake via the reduction of plant N demand caused by the reduction in the relative growth rate (Kafkafi and Bernstein, 1996). Some authors reported that salinity affected negatively the relative growth rate of plants due to a decrease in photosynthesis which influence the internal N demand and the rate at which N is taken up by roots. Soil N contribution to plants was also reduced in saline conditions, which indicate the effects of salinity on N fixation and transformation of organic N to mineral N (Ashraf et *al*., 2018).

2.1.2. Salinity effect on N assimilation

N assimilation into carbon skeletons is of great importance for plant development and growth, particularly in saline environment (Lea and Mifflin 2003). Furthermore, many nitrogenous

compounds derived from N assimilation such as amino acids, amines and proteins are assumed to be essential for plant resistance to salinity and believed to be involved in osmotic adjustment and scavenging of ROS (Ashraf el *al*., 2018). Once absorbed and transported, nitrate (NO_3^-) can be stored in the vacuole or metabolized in root or leaf cells. To be metabolized NO_3^- must be first reduced to ammonium (NH_4^+) prior to its incorporation into organic form (Zhang et *al.,* 2014). NO_3^- reduced to NH_4^+ in two successive enzymatic steps: NO_3^- reduction and NO_2^- reduction. During N assimilation, salinity affects NO_3^- uptake and transport rather than NO_3^- reduction (Hossain et *al*., 2012).

2.1.2.1. Nitrate (NO_3^-) reduction

This step takes place in the cytoplasm where Nitrate (NO_3^-) is reduced to NO_2^- by nitrate reductase cytoplasmic enzyme (NR) (Figure 4), using pyridine nucleotide as source of reductant (Cao et *al.,* 2008). NR is a soluble enzyme generally located in the cytosol of root and leaf cells (Oaks and Hirel, 1985) and catalyzes the reduction of NO_3^- to NO_2^- via the transfer of two electrons from a cofactor (Meyer et *al.* Stitt, 2001). In higher plants, there are two isoforms of NR differentiated by the reducer power donor: NADH or NADPH (Rouzé and Caboche, 1992). NADH/NR is the most common isoform in higher plants. It is an enzyme localized in leaf chloroplasts, root plastids and other non-chlorophyllous organs. The presence of the substrate (NO_3^-) rapidly induces (in few hours) the expression of genes encoding NR (Patterson et *al*., 2010). Its activity is stimulated by light, CO_2, water and NO_3^-. Different mechanisms allow its regulation to prevent the accumulation of toxic NO_2^- at high concentration in cells (Lillo, 2008). NO_3^- assimilation seems to be sensitive to salinity in different crops such as Vigna unguiculata, Anacardium occidentale, Triticum durum, Morus alba L., Arabidopsis thaliana, Glycine max L. (da Silveira et *al*., 2003; Carillo et *al*., 2005; Surabhi et *al.* 2008; Maaroufi-Dguimi et *al*., 2011; Queiroz et *al*., 2012).

In fact, plants subjected to salinity generally exhibit restriction in NO_3^- acquisition. As a direct result, these plants represent a low NO_3^- accumulation in tissues, notably in leaves and stems which subsequently decrease NR activity (Ashraf et *al*., 2018). The decrease of NO_3^- uptake and flux into the stem which inhibits leaf NR activity and NO_3^- assimilation under salinity was reported by Parida and Das (2004) and Wang et *al*. (2004), whereas the decrease of root NR activity is less frequently (Meloni et *al*., 2004). The fact that NR activity was more affected in leaves than in roots, despite the large reduction in NO_3^- concentrations in both organs, suggests that NR activity is regulated separately by NO_3^- availability in each plant organs (Ashraf et *al.*, 2018).

NO_3^- is a signal affecting NR expression and activity, and its decrease may have strict effect for whole-plant NO_3^- assimilation. It has been suggested that cytosolic NO_3^- protect the NR enzyme against the action of proteases and/or inhibitors (Ashraf et *al*., 2018). Under saline conditions, NR activity could be reduced because of enzyme degradation or inactivation, decrease in gene expression and NR protein synthesis (Carillo et *al.,* 2005). Under salinity, various assimilates like sugars and amino acids can be included in the regulation of NR expression in plants (Matt et *al*., 2001).

2.1.2.2. NO_2^- reduction

Since NO_2^- is extremely reactive, plant cells immediately transport it from the cytosol into leaves chloroplasts and roots plastids, where it is reduced to NH_4^+ by Nitrite reductase (NiR) (Rosales et *al.,* 2011) (Figure 4). NiR catalyzes the reduction of NO_2^- to NH_4^+ with the addition of 6 electrons provided by ferredoxin to NO_2^- (Meyer and Stitt, 2001). This enzyme is encoded by single gene and located in the chloroplasts of leaves' cells and plastids of root cells (Deroche, 1983); its activities are dependent on the carbohydrate content in the root tissues, as a sources of reducing power and carbon skeletons.

It has been found that salinity reduces the nitrate influx and reduction which inhibit NR and NiR activities in plants (Flores et *al*. 2004). Co-regulation hypothesis between NR and NiR was confirmed by Ogawa et *al.* (2000), in fact co-regulation is crucial to avoid NO_2^- toxicity and prevent electrons waste by NR and NiR activities. NiR activity is comparatively less affected by salinity than NR activity, which could be due to more stable nature of NiR proteins (Bray, 1997). In the same context, Debouba et *al*., (2006) demonstrated that NiR activity was observed to be much higher than NR in tomato.

2.1.2.3. Ammonium assimilation

NH_4^+ can have several origins: It can be absorbed directly by the roots, or produced by reductions of NO_3^-, or from atmospheric N. It can be also derived from the photorespiration and catabolism of certain amino acids or other compounds rich in nitrogen (Ashraf et *al*., 2018). NH_4^+ is toxic compound to plant cells and its uptake cause proton extrusion, cytosolic pH disruption, and uncoupling of photophosphorylation. Therefore, a rapid assimilation into non-toxic organic N compounds is a vital necessity for plants. This can be achieved by the incorporation of ammonium into amino acids through the combined action of two key enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT). Other enzymes, such as glutamate dehydrogenase (GDH) or asparagine synthetase (AS) can also play an important role in NH_4^+ assimilation (Annunziata et *al*., 2017) (Figure 4).

GS enzyme is known by its strong affinity for ammonium and its ability to integrate into organic molecules. It has chloroplastic and cytosolic forms (Deroche, 1983). GS1 is the cytosolic and plurigenic form, it is present generally in the roots and non-photosynthetic tissues. GS2 is the chloroplasic and monogenic form. It is present generally in the photosynthetic tissues, especially young leaves (Merigout, 2006). The relative proportions of these two isoforms varied according to species and stage of development. There are two types of glutamate synthase GOGAT (Glutamine 2-Oxo Glutarate Amino Transferase) localized in chloroplasts and distinguished by the electron donor: Ferredoxin (Fd-GOGAT) or NADH (NADH-GOGAT). Fd-GOGAT is generally present in leaf chloroplasts and accounts for 95% of GOGAT activity, NADH-GOGAT is active mainly in the plastids of non-photosynthetic tissues such as roots (Masclaux-Daubresse et *al.*, 2010). Their tissue localizations suggest a predominant role of Fd-GOGAT in the primary assimilation of ammonium, whereas, NADH-GOGAT is specifically involved in the reassimilation of photorespiratory ammonium process (Merigout, 2006). GS and GOGAT are the main enzymes involved in NH_4^+ assimilation (Esposito et *al.*, 2005; Wickert et *al.*, 2007). Through the GS/GOGAT pathway, NH_4^+ is incorporated into glutamine (Gln) by GS which is then converted to glutamate (Glu) by GOGAT (Lancien et *al.* 2000). Amide group (NH_2) of glutamine is transferred to a ketonic acid (α-ketoglutarate) under the action of GOGAT (Deroche, 1983); thus, two molecules of glutamate are formed, one is used as substrate for GS to form glutamine and will and replenish the GS / GOGAT cycle. The second is an N organic source for transaminating reactions that lead to the synthesis of all the amino acids namely aspartate involving the enzyme Aspartate Aminotransferase (AAT), and Asparagine via Asparagine Synthetase (ASN). Glutamine, glutamate, aspartate and asparagine are compounds that are mainly used for the transport of N and its translocation from source to sink (Hirel and Gaillais, 2013). GDH pathway synthesizes Glutamate from 2-oxoglutarate and NH_4^+ (Lancien et *al.*, 2000). Generally, GS activity is higher than GOGAT activity under normal conditions (Meng et *al.*, 2016). However, salinity decreases the activities of both GS and GOGAT enzymes, and it was demonstrated that GOGAT is more sensitive to salinity effects, especially at high salt concentration which suggests GOGAT that is the more inhibiting factor in NH_4^+ assimilation in saline environment (Khadri et *al*., 2001; Kawakami et *al.*, 2013). In the same context, Wang et *al*., (2012) reported that the decrease in NH_4^+ assimilation under salinity is probably due to the down regulation of the genes OsNR1, OsGS1; 2, OsGS2, and OsFd-GOGAT.

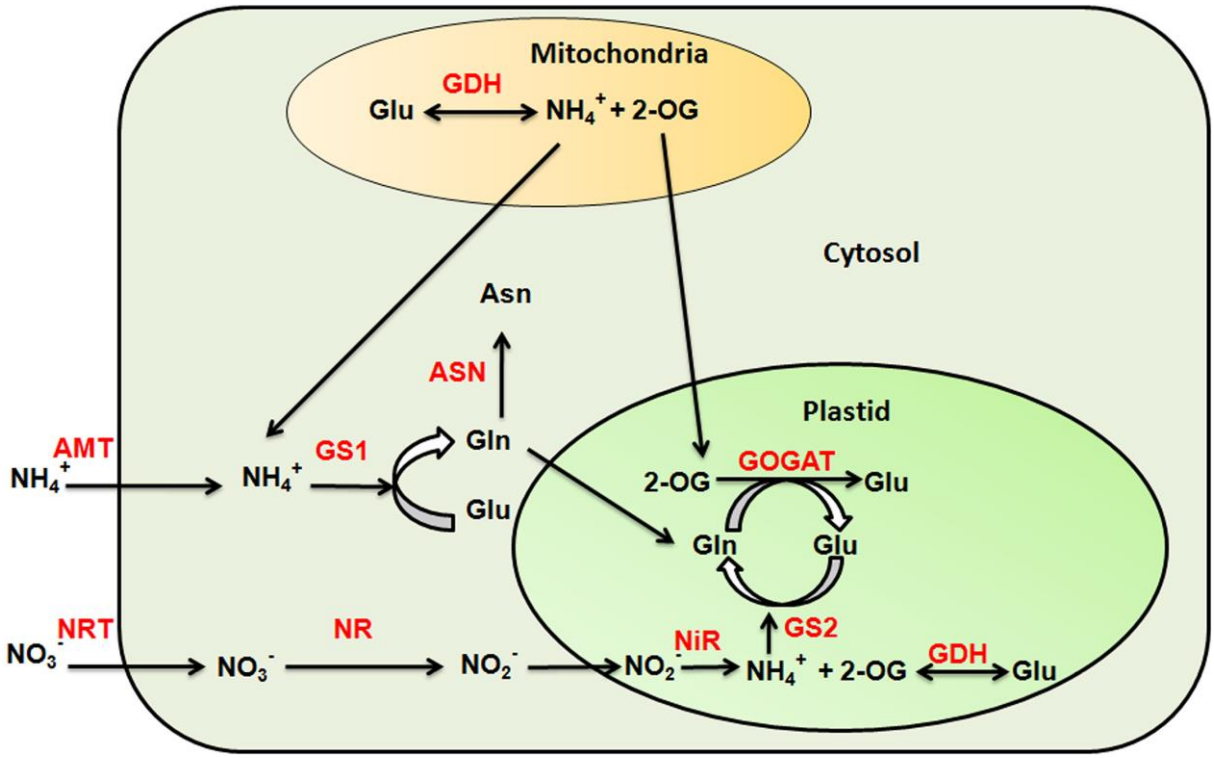

Figure 4: Nitrogen uptake and assimilation in plants. The uptake of NO_3^- and NH_4^+ ions is insured by NO_3^- transporters (NRT) and NH_4^+transporters (AMT) (Goel et *al.*, 2015).

Into the cell, NO_3^- is reduced to nitrite (NO_2^-) by nitrate reductase (NR) enzyme. In the plastid, NO_2^- is reduced to NH_4^+ by nitrite reductase (NiR) enzyme. The NH_4^+ is then incorporated into amino acid by glutamine synthetase (GS) and glutamate synthase (GOGAT) via GS/GOGAT cycle. The enzymes glutamate dehydrogenase (GDH) and asparagine synthetase (ASN) can also play an important role in NH_4^+ assimilation and the synthesis of glutamine (Gln), Glutamate (Glu) and Asparagine (Asn).

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It is believed that the GS/GOGAT cycle is the major pathway for NH_4^+ assimilation under normal conditions. However, the decrease in GS/GOGAT activities under salinity limits the glutamine and amino acid production, and increases NH_4^+ accumulation which can activates the alternative pathway for NH_4^+ assimilation: the GDH pathway. The accumulation of NH_4^+ in plant cells and the activation of GDH pathway through the trigger of GDH activity has been observed in several plants such as Phaseolus vulgaris, Oryza sativa L., Triticale hexaploide Lart, Solanum lycopersicum (Ashraf et *al*., 2018). Salt stress may increase leaf protein catabolism and inhibit NH_4^+ assimilation which results in high amount of free amino acids and NH_3. The excessive NH_3 can be detoxified by the acceleration of N assimilation via the usual pathway (GS/GOGOAT cycle) or by the reinforcement of the GS/GOGAT pathway by further NH_3 utilizing reactions (GDH pathway) (Wang et *al*., 2012). Also it was reported that the excess of Na^+ and Cl^- anions could change NH_4^+ assimilation pathway by inactiving the GS/GOGAT cycle and stimulating of GDH pathway (Surabhi et *al*., 2008). Two types of responses have been typically differentiated on the basis of enzyme activities alteration under salt stress: (i) early responsive enzymes like NR, NAD-GDH in leaves and Fd-GOGAT in leaves and roots. These enzymes change as early as 24 hour after adding salts. (ii) The late responsive enzymes like leaves NADH-GOGAT, roots NAD-GD, NiR, GS and NADH-GDH in leaves and roots. These enzymes are generally affected at least 4 days after salt addition (Ashraf et *al*., 2018).

Chapter I: Yield and N uptake distribution pattern in contrasting barley genotypes (*Hordeum vulgare* L.) grown in Mediterranean arid environment

This chapter is prepared for submission in journal of Soil Science and Plant Nutrition

1. Abstract

Nitrogen (N) uptake and distribution pattern contributes largely, in saline environment, to grain yield and grain quality. The objective of this study was to investigate genotypic differences for N uptake and its partitioning, in four contrasting barley genotypes (two are tolerant to salinity: "100/1B" and "Souihli", while "Barley medenine" and "ICARDA20" are susceptible) grown for 3 years under eight combinations of N supply (0, 50, 100, and 150 kg N/ha) and water salinity (1.8 and 9,2 dS/m) in arid region. N supply –despite the disruption caused by salinity- improved grain and biomass yield and N accumulation in different plant part (grain, straw, awns) with a greatest enhancement under salinity. The reach of grain yield (GY) plateau was significantly dependent on genotypes and saline conditions. Providing more N supply beyond 100N can decrease GY depending on genotypes. Under 150N, excessive N remain in straw in "ICARDA20" (42% of the total N uptake compared with 27% under sub 150N), while it was allocated to awns in "100/1B" (13,5% of the total N uptake compared with 8.5% under sub 150N). Interestingly, "Souihli" showed a luxury accumulation of excessive N: It absorbs N efficiently under all conditions and convert it into grain protein. Grain N content contributed by 60% to the total N uptake, while straw and awns represent 30% and 10% respectively. The high N accumulation in "Barley Medenine" awns associated with the low grain N accumulation altered N allocation to the spike and decreased its yield.

Key words: salinity, Nitrogen supply, Barley, N uptake, N distribution pattern, luxury N accumulation.

2. Introduction

Nitrogen (N) is considered as the most important nutrient influencing plant growth and productivity (Singh et *al*., 2016). An effective and adequate management of nitrogen is more needed than any other input to enhance crop yield (Malhi et *al*., 2001).

Nutrient uptake can be affected by various factors such as salinity of soil and water which are a serious worldwide problem (Munns and Tester 2008). Salinization is more accentuated in arid and semi-arid climates on account of high evapotranspiration (Hammami et *al*., 2017).

Salinity leads to an ion imbalance in the soil because of the accumulation of Sodium (Na^+) and chloride (Cl^-), known to compete nitrogen in its ammonium (NH_4^+) and nitrate (NO_3^-) forms (Abdelgadir et *al.* 2005, Dluzniewska et *al*. 2007). Consequently, salt stress perturb N metabolism essentially due to reduced N uptake (Ashraf et *al.* 2017). Also, the change in soil water potential and root osmotic potential with the reduction of transpiration rate caused by salinity can reduce water absorption and affect the accessibility to soil Nitrogen (Ehlting et *al.*, 2007; Gessler et *al*., 2005; Debouba et *al*., 2007; da Silveira et *al.*, 1999). Some other authors indicated that the reduction of plant growth under salinity induce a lower N uptake needed for a plant (Ullrich, 2001).

Previous studies have shown that the interaction between salt stress and nitrogen nutrition is very complex and not well understood (Irshad et *al.*, 2002), the particular complexity in this interaction is the two-way relationship. In another word, as salinity inhibits plant development and nitrogen metabolism, evenly Nitrogen fertilization enhances plant nutrition and could alleviate the negative effect of salinity.

In arid environment, saline soil is often suffered from multiple stresses such as N deficiency (Song et *al*., 2019). In this area, barley (*Hordeum Vulgare* L.) is an essential source of human and animal feed. Furthermore, it is a major crop replacing wheat view its tolerance to salinity (Johnson and Flower, 1992). In the same plant species, there is a natural variation between genotypes. Therefore, to maximize yield in saline environment, and minimize production cost and environmental pollution caused by nitrate leaching, nitrogen efficient genotypes tolerant to salinity are of real interest for researchers. Total N uptake, is an important genetic trait that contribute to Nitrogen use efficiency and explain its main variation (Xu et *al*., 2012).

The empirical findings on the interaction between salinity and Nitrogen nutrition in the field are limited and less well studied; furthermore less effort has been invested in barley compared with other cereals. Therefore, to investigate genotypic differences for N uptake and its partitioning in different plant parts in four contrasting genotypes of barley, a field experiments were conducted on 3 years under different combinations of nitrogen supply and water salinity

in a Mediterranean, arid region. The four Barley genotypes were selected for their different sensitivity to salinity. In addition, the agronomic performances of different genotypes and the optimal nitrogen supply must be provided in such environment were performed.

3. Materials and methods

3.1. Field site and climates conditions

Field experiments were conducted, during three cropping seasons (2016–2017, 2017–2018 and 2018-2019) at the arid area El Fjé-Medenine (33°26′54”N,10°56′31”E) localized in the South East of Tunisia (Figure 1).

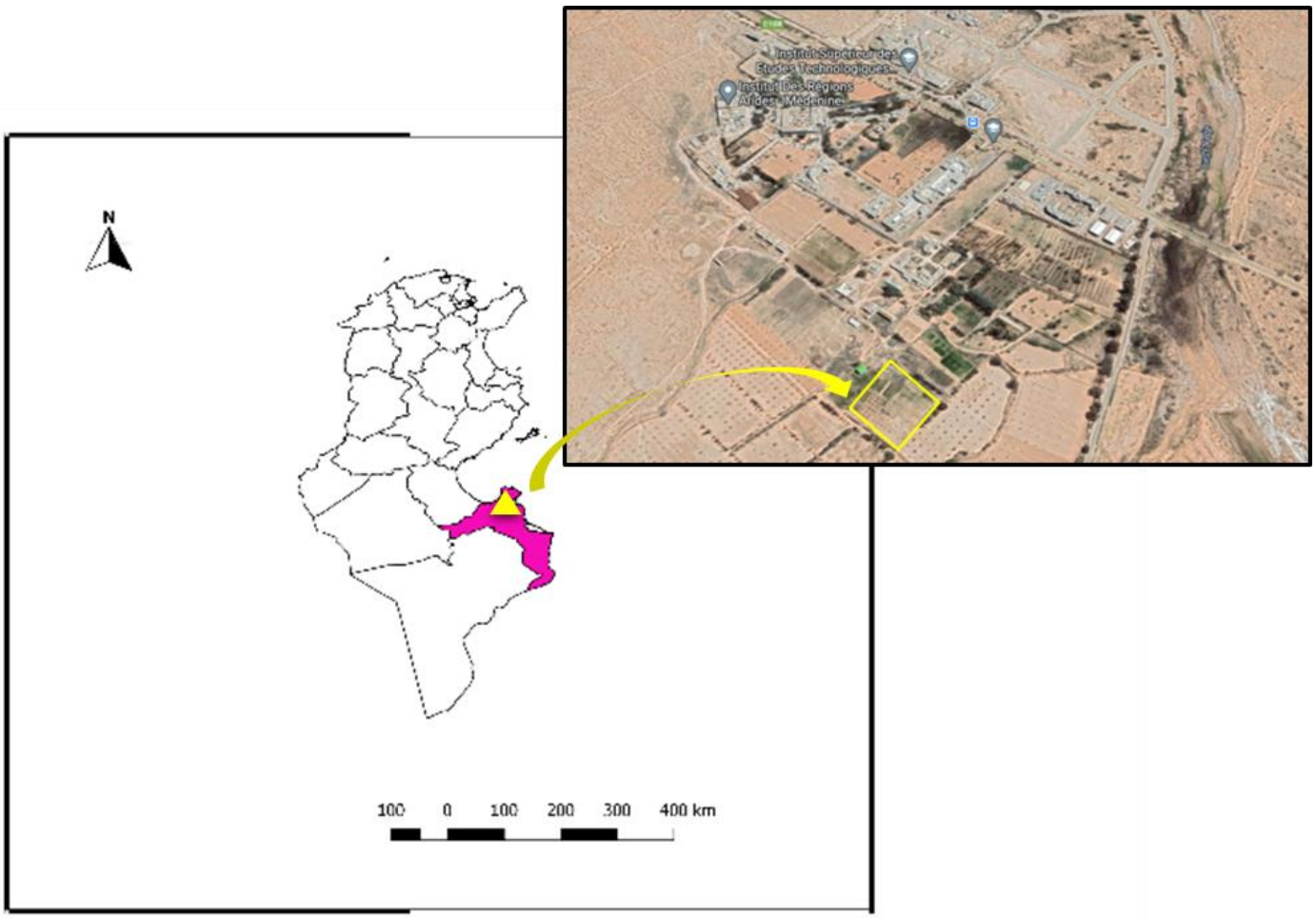

Figure 1: Location of the study area. The red color defines Medenine governorate (QGIS), and the yellow color represents the study area (Google Earth Pro, 2021).

The site El Fjé is characterized by an arid climate with irregular annual rainfall. According to Climate-data-org, the average of precipitation in Medenine is around 131 mm/year. To better characterize climatic conditions, the amount and distribution of rainfall, maximum and minimum temperatures data were collected from a meteorological station located in the experimental site (Figure 2). The maximum and the minimum temperatures are relatively

comparable across the three growing seasons, while the amount of rainfall was notably different especially for the second year (Y2) characterized by a high rainfall value of 290 mm. The first year (Y1) is marked by low rainfall value of 156 mm while the third year (Y3) is characterized by an intermediate rainfall amount of 167 mm.

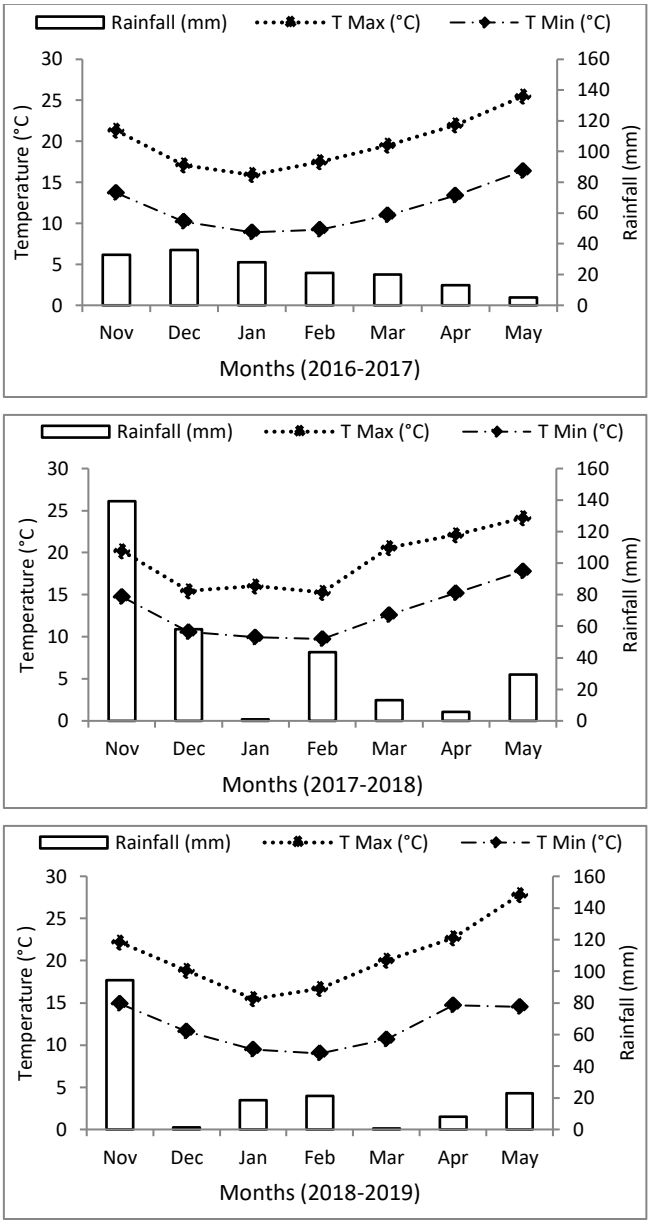

Figure 2: Rainfall, temperatures for the cropping season 2016-2017 (Y1), 2017-2018 (Y 2) and 2018-2019 (Y 3).

3.2. Soil characteristics

The experimental site is classified as sandy soil. It is composed by 4,5% clay, 14,8% silt, and 80,7% sand, it is also characterized by low organic matter content (0.9%) with high electrical conductivity (EC) of 2,8 mS/cm and pH=7,7. The soil contained a low content of active limestone (5%) and assimilable phosphorus (9 ppm). Soil contained a low average of

ammoniacal and nitric nitrogen content of 7.5 and 8.5 mg/kg respectively. Soil exchangeable bases content is relatively low: 15 and 60 ppm respectively for Na^+ and K^+. Soil apparent density is around 1.2. Initial nitrate and ammonium soil content for the different years is summarized in table 1.

Table 1: Nitrate and ammonium content in the soil.

	Initial N-NO_3^- content (kg N ha^{-1})	**Initial N-NH_4^+ content (kg N ha^{-1})**
2016-2017 (Y1)	**44.5**	**33.5**
2017-2018 (Y2)	**35**	**57**
2018-2019 (Y3)	**20**	**38**

Y1: Year 1 ; Y2: year 2 ; Y3: year 3

3.3. Plant material and experimental design

Four Barley genotypes were grown up on field during three years (Y1, Y2, Y3). These genotypes have a contrasting behavior in their response to salinity (Hammami et *al*., 2017). The two genotypes ("100/1B" and "Souihli") are considered as tolerant, while "Barley Medenine" and "ICARDA20" as susceptible genotypes, their characteristics are presented in Table 2. These genotypes were sown by hand at a density of 200 seeds m^{-2}).

The experimental design was a split-split plot. Trail field was divided into two sub-plots, and three blocks were designed perpendicularly to the sub-plots. In each block two irrigation treatments were used with two salinity levels: low and high salinity. Each individual plot consisted of 6 rows spaced by 20 cm with 2 m length.

Table 2: characteristic of tested Barley genotypes.

Genotypename	**Tolerance to salinity**	**Type**	**Location**	**Soil salinity in the origin area(dS m^{-1})***
"100/1B"	**Tolerant**	**Landrace**	**Oman**	**2**
"Souihli" (beldi/sahli)	**Tolerant**	**Landrace**	**Tunisia:Mahdia,ksas**	**2.7**
"Barley Medenine"	**Susceptible**	**Landrace**	**ICARDA/Tunisia**	**1.3**
"ICARDA20"	**Susceptible**	**Cultivar**	**ICARDA**	**0.3**

*Soil salinity (in test performed using Electrical Conductivity 1:5 ("EC one-to-five")

3.4. Nitrogen and Irrigation Management

Four N fertilizer rates were applied: 0, 50, 100, and 150 kg N/ha in three applications: 30 % at early tillering (Z13), 40% at stem elongation (Z16) on and 30% at the second node stage (Z32). N fertilizer was applied as ammonium nitrate (33,5%). The four genotypes were irrigated with

two water salinity levels 1.8 (control: low saline water) and 9,2 dS/m (high saline water). In order to obtain homogeneous water supply, irrigation was conducted through a drip system: line source emitters 33 cm interspaced was installed at each planting row. Barley water requirement was provided from planting to grain filling according to climatic and soil data.

3.5. Sampling and data analysis

Plots were hand-harvested at maturity to determine grain yield and its components. In order to avoid the edge effect, only the four central rows were used for yield assessment. Above-ground biomass was separated into straw and spikes. Grains were obtained using a laboratory thresher (Wentersteiger, LD-180, Germany) and awns were collected for N determination.

All Samples were oven dried for 48h at 65°C before weighting. All samples were ground by a rotor mill to obtain a fine powder used for N analyses. N concentration was determined by the Standard Kjeldahl procedure and N concentration was measured according to the Cataldo et *al.* (1974) method. N content were calculated by multiplying N concentration by dry weight.

Total N in plants at maturity was estimated as the sum of grain N content plus straw N content plus awns N content. Grain protein content (GPC) was measured in dry grains by the Kjeldahl method and then converted to crude grain protein using a conversion factor of 5.7 (Barraclough et *al.*, 2010).

3.6. Statistical analyses

Statistical analyses were done using R software (R-64 3.6.1). To evaluate the main effect of genotypes, Nitrogen rate, and salinity and their interactions, ANOVA analysis was performed. The significance of factor was determined according to p-values. Duncan test ($p < 0.05$) was used for means comparison test. Multiple linear regression analysis (stepwise) was conducted to identify best traits related to Nitrogen uptake and explaining grain yield as a dependent variable. The relationship between main variables contributing to grain yield was studied through correlation analyses. To design figures GraphPad Prism 8 program was used.

4. Results

4.1. Weather conditions and crop productivity

Crop productivity evidently under salinity was noticeably influenced by the amount of rainfall (Table 3). In the highest rainfall year (Y2), the mean of both grain and biomass yield under salinity was markedly high: 4 and 5.8 T/ha respectively while the lowest rainfall year (Y1) production decreased to 1.9 T/ha of GY and 1.8 T/ha for BY. An intermediate mean grain and biomass yield of 2.6 T and 5.4 T/ha under salinity were observed in Y3. Similarly, in saline conditions the highest values were noticed in Y2 for grain number (9694 grain/m^2) and total N

uptake (72 KgN/ha) and the lowest was observed in Y1 (4363 and 42 KgN/ha respectively). It is important to underline that these parameters were slightly influenced under low saline condition across the years.

Table 3: Effect of cropping seasons on Grain yield (GY), Biomass yield (BY), Total N uptake (Tot Nup), and Grain number (Grain nb) under high and low saline conditions in four Barley genotypes treated by different nitrogen rate (0, 50, 100 and 150 N).

Year	Rainfall	salt stress	GY (T/ha)	BY (T/ha)	Tot Nup (KgN/ha)	Grain nb
2016-2017 (Y1)	**156 mm**	**low salinity**	**3.3^{c}**	**3.8^{e}**	**105.3^{a}**	**7735.6^{c}**
		high Salinity	**1.9^{e}**	**1.8^{f}**	**42^{d}**	**4362.8^{e}**
2017-2018 (Y2)	**290 mm**	**low salinity**	**3.9^{ab}**	**7.4^{b}**	**104.6^{a}**	**9803.1^{ab}**
		high salinity	**4.0^{a}**	**5.8^{c}**	**71.7^{c}**	**9694.3^{b}**
2018-2019 (Y3)	**167 mm**	**low salinity**	**3.9^{ab}**	**8.2^{a}**	**98.8^{b}**	**10199.4^{a}**
		high salinity	**2.6^{d}**	**5.4^{d}**	**69.4^{c}**	**6866.2^{d}**

4.2. Grain yield and its component

The analysis of variance (ANOVA) showed a significant variation among year, genotype, salinity and Nitrogen levels for the all parameters (Table 4).

ANOVA revealed a clear difference between mean grain GY. It was varied between 2.4 and 4.8 (t/ha) under low salinity, and 1 and 4.5 t/ha under high salinity conditions. On average, the mean of reduction caused by saline irrigation was 0,85 t/ha, which was observed especially for the two nitrogen levels 0N,50N.

GY was enhanced significantly as N supply was increased to 100N for all genotypes, except the improved "ICARDA20" which reach a maximum yield and a plateau with 50N under low salinity (Figure 3). However, more supply of N showed different responses depending on genotypes and salinity. In fact, under low salinity condition, GY was decreased by 19.15% and 11% with increasing N supply to 150N in the two genotypes"100/1B" and "ICARDA20" respectively; while the genotypes "Souihli" and "Barley Medenine" did not show any significant variations in GY between 100N and 150N. So, applying more than 100N in low-saline condition was disadvantageous. Whereas under high saline irrigation, GY of the genotypes cultivated under 150N did not show any decrease: "100/1B" and "Barley Medenine" displayed a continuous GY rise with further N addition, while "Souihli" and "ICARDA20" reach the plateau under 100N. In that case, 150N application improved GY only for the two landraces "100/1B" and "Barley Medenine".

Table 4: Analysis of variance showing mean square of different trait for the 4 genotypes under different N and saline treatments.

	df	grain yield (GY)	Biomass yield (BY)	Grain N content	Straw N content	Awns N content	Total N uptake	Grain protein concentration	TKW	Grain number
Year (Y)	2	43.23***	485.2***	445**	4119***	435.4***	5468***	153.31***	534.0***	341322242***
Variety(V)	3	12.40***	118.5***	2283***	1616***	25.8**	7287***	6.70***	1356.7***	44708982***
Salinity (S)	1	53.05***	348.9***	39584***	16099***	924.1***	126905***	202.73***	9.0.	371537266***
Nlevel (N)	3	61.28***	209.3***	15010***	7732***	419.6***	52799***	6.84***	14.2**	380910290***
Y *V	6	1.35***	9.4***	362***	114***	29.6***	575***	1.74	108.1***	6843312***
Y *S	2	20.14***	8.1***	5290***	860***	56.6***	8423***	51.23***	77.8***	84207671***
V*S	3	0.32	1.3.	100	144**	27.8**	665**	4.03*	2.3	3841687*
Y*N	6	3.12***	22.7***	845***	629***	93.6***	3448***	7.88***	36.6***	27764771***
V*N	9	0.91***	1.5**	278***	143***	18.1**	543***	2.22*	2.8	5446815***
S*N	3	8.43***	2.5**	398***	253***	28.8**	752***	10.32***	13.2**	41927509***
Y*V*S	6	0.80***	3.0***	347***	364***	19.8**	645***	1.22	12.8***	6478977***
Y*V*N	18	0.18	1.4**	97.	114***	26.4***	258**	0.92	4.6	1496406
Y*S*N	6	4.05***	1.3*	278***	377***	85.7***	443**	2.30.	8.5*	18512644***
V*S*N	9	1.03***	2.4***	253***	240***	8.8	594***	0.99	4.4	4946301***
Y*V*S*N	18	1.10***	2.1***	284***	98***	30.5***	623***	1.24	4.4	8657942***
Residuals	192	0.18	0.5	64	27	6.0	126	1.13	3.1	1218667

*: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$

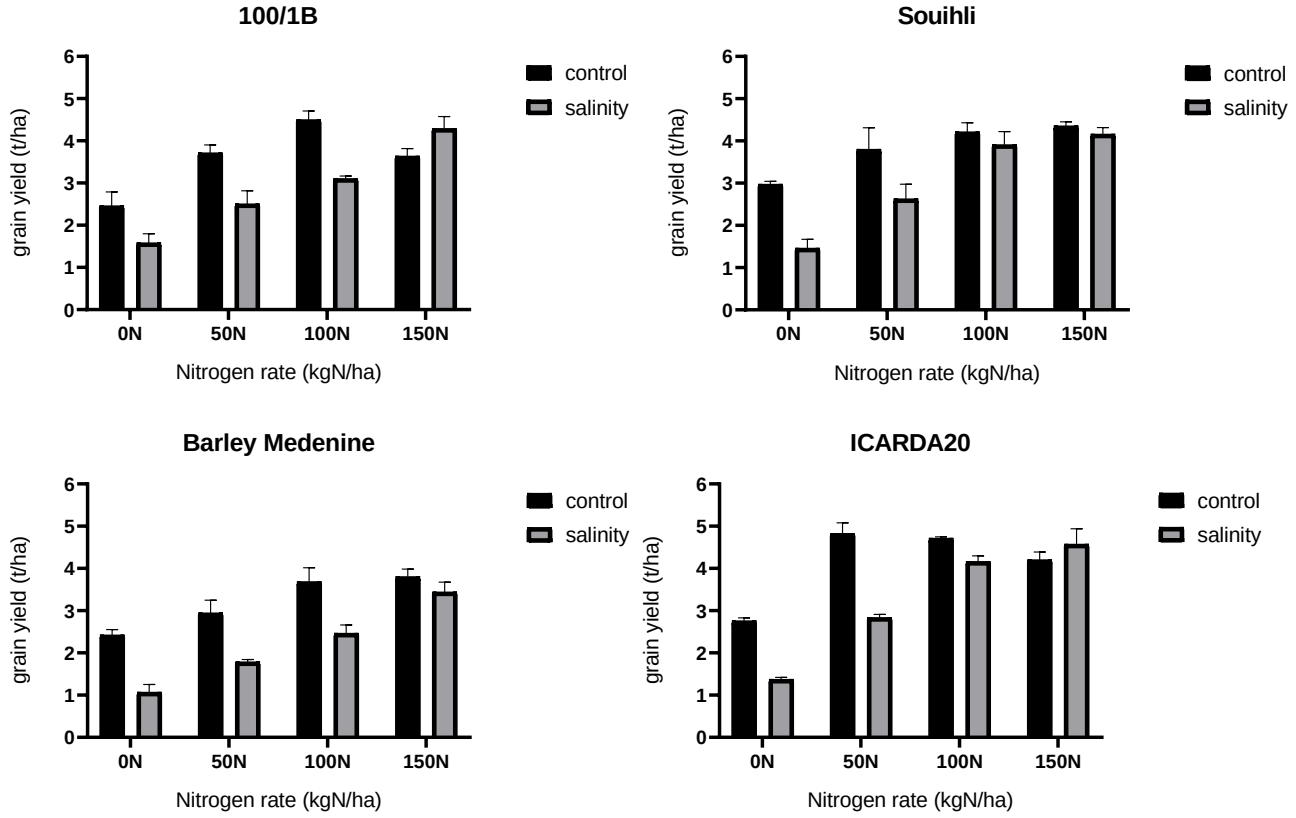

Figure 3: Grain yield (t/ha) in different genotypes grown under 0N, 50N, 100N and 150N treatment under control (black) and saline irrigation (grey).

Grain number was significantly reduced by 24.6% under saline irrigation. All genotypes under optimal or stressful conditions reacted positively and similarly to the N supply, except for the genotypes "100/1B" and "ICARDA20" under 150N application. These two genotypes recorded a significant reduction of grain number under 150N rate in low salinity condition. These results suggest the closed relation between GY and grain number per area.

Thousand kernel weight (TKW) ranged widely across the genotypes: from 36.4 g for "Barley Medenine" to 46.5g for "Souihli", while "ICARDA20" and "100/1B" showed 40.4g and 38.4 g respectively. Globally, N application enhanced grain weight compared to starved plants but no significant difference was found between 50N,100N and 150N.

4.3. Biomass yield

Biomass yield at maturity ranged between 3 and 6 t/ha in low salinity condition without N supply; 1.3 and 3 t/ha in unfertilized plants grown under high salinity conditions (Figure 4).

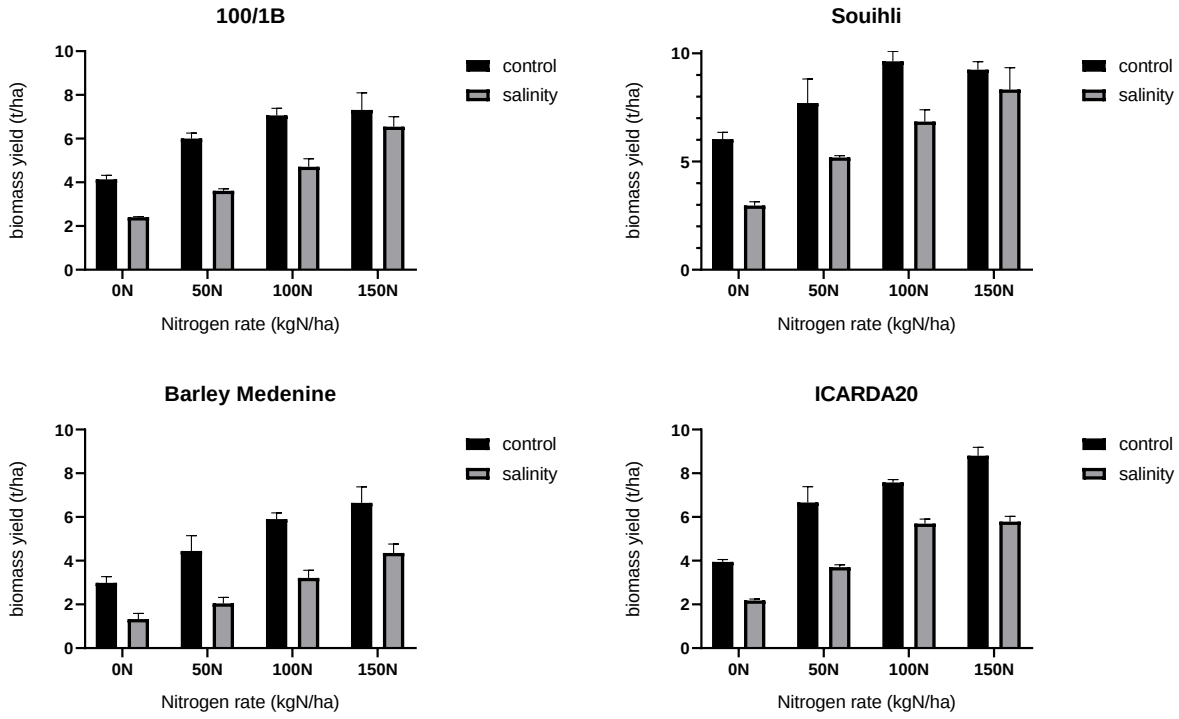

Figure 4: Biomass yield (t/ha) in different genotypes grown under 0N, 50N, 100N and 150N treatment under control (black) and saline irrigation (grey).

Applying N fertilizer increased significantly biomass yield which varied in fertilized plots between 4.4 and 9.6 t/ha in low saline condition; 2 and 8.3 under salinity. Biomass yield increased by 34%, 49% and 55% with increasing N fertilizer to 50N, 100N and 150N

respectively (Figure 4). Results showed that no significant effect was revealed under low salinity between 100N and 150N for the two tolerant genotypes "100/1B" and "Souihli".

Overall, saline irrigation caused a 33% decrease in biomass yield (from 6.5 to 4.3 t/ha). Regarding reduction biomass caused by salinity, "100/1B" genotype was the most stable and the less affected: 1.8 t/ha of reduction, while it was around 2.35 t / ha for the other genotypes.

Whatever saline and nutritional conditions, "Souihli" produced higher biomass than other genotypes while "Barley Medenine" recorded the lowest biomass yield and the least beneficiary from N addition.

4.4. N uptake pattern and Grain Protein Concentration (GPC)

Salinity decreased the total amount of absorbed N by 41% (from 103 to 61 KgN/ha). Despite that the highest N uptake was observed in "ICARDA20" under low saline irrigation, this last with "Barley Medenine" were the most affected by salinity (45% of reduction), while it did not exceed 37% for the two tolerant genotypes "100/1B" and "Souihli" (Figure 5).

N treatment improved significantly total N uptake. Under low saline condition, the highest improvement was registered in "ICARDA20" (52%) while it was around 42% for the other genotypes; 50N, 100N and 150N enhanced total absorbed N by 35%, 47% and 51% respectively compared with unfertilized plants. N fertilization induced a greater N uptake enhancement under salinity, in fact, 50N, 100N and 150N caused 40%, 57% and 65% of improvement respectively compared with 0N treatment. In saline conditions, "Souihli" and "ICARDA20" did not show any significant difference between 100 and 150 KgN/ha and they showed an important total N improvement of 60% in response to N fertilization while it was 57% in "Barley Medenine" and 49% in "100/1B". Interestingly, "Souihli" showed its capacity to absorb N under all saline and nutritional conditions, on the other side, "Barley Medenine" showed the lowest values.

4.4.1. Grain N content

N supply improved significantly grain, straw and awns N content. Under low saline irrigation, 50N improved grain N content by 34% while 100N and 150N which were statistically similar induced 44% enhancement compared with unfertilized plants (Figure 5). The beneficial effect of N fertilization were more evident under salinity, in fact 43% , 59% and 66% were the improvement for 50N, 100N and 150N respectively, when compared with 0N. Regardless salinity, under 150N, "Souihli" accumulate the highest grain N content among all genotypes.

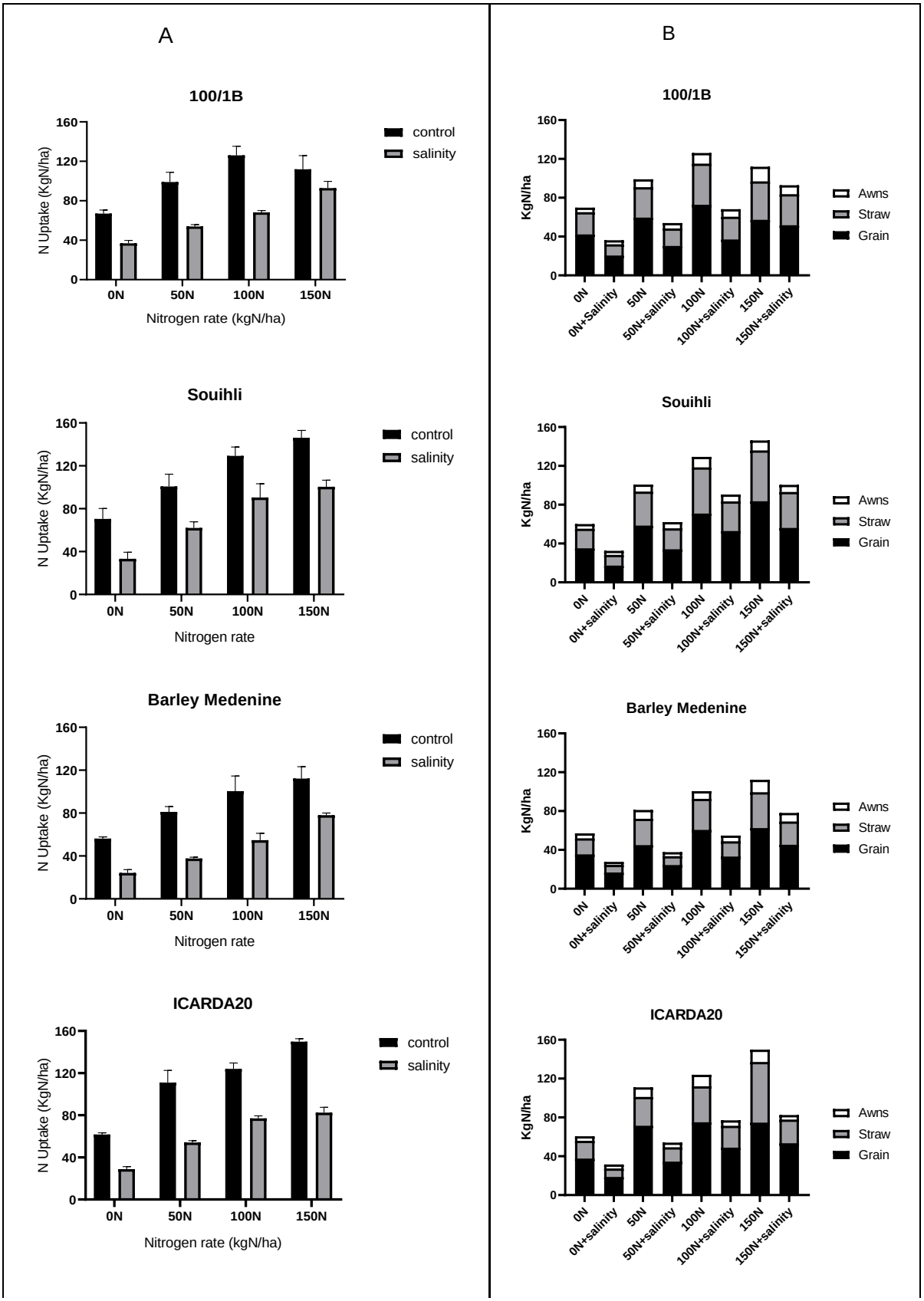

Figure 5: Total N uptake (A) and portioning between plant parts (B) in different genotypes grown under 0N, 50N, 100N and 150N treatment under control and saline irrigation

Contrariwise, under 150N, when irrigated with low saline water, "100/1B" showed 21% of reduction of grain N accumulation compared with 100N, which can explain the reduction of total Nuptake. Under low salinity, "ICARDA20" showed a benefit to enhance grain N content from low N supply (50N), then any significant difference was observed between 50N, 100N and 150N.

Correspondingly, to total N uptake under salinity, 100N and 150N were statistically similar in "Souihli" and ICARDA 20 and markedly different in the two landraces "100/1B" and "Barley Medenine". Furthermore, the lowest grain and straw content N was observed in "Barley Medenine" which is in correspondence with total Nuptake. Whatever salinity, grain N represented the major N pool and contributed by 60% to the total Nuptake, while straw and awns represent 30% and 10% respectively.

4.4.2. Straw N content

Regardless salinity and N application, "Souihli" showed the highest N straw content, followed by "100/1B" and ICARDA 20 (Figure 5). Under low saline condition, 150N increased markedly straw N content from 28 KgN/ha to 62 KgN/ha in "ICARDA20" which is marked by its low N straw accumulation. This indicates that the excess of N in this genotype remains in straw and is not remobilized to the grain.

4.4.3. Awns N content

Under 0N, 50N, 100N, awns N content was statistically similar in all genotypes irrigated with low saline water. N accumulation in awns increased significantly under 150N in "100/1B" (15 KgN/ha) compared to sub 150N dose (8,5 KgN/ha). The other three genotypes accumulated only 12 kgN/ha under 150 N supply (Figure 5). Whereas, 12 Kg/ha of N accumulated in awns is considered a high amount in "Barley Medenine" because of its low total N accumulation. Taken together, under high N and low salinity, N accumulation in awns contributed by 13,5% and 11,5% to the total N in plants for "100/1B" and "Barley Medenine" respectively, while it was around 7,7% for "ICARDA20" and "Souihli". Likewise, under salinity, "100/1B" and "Barley Medenine" fed with 150N showed the highest awns N content: 9 KgN/ha compared with 6 KgN/ha for "ICARDA20" and "Souihli". Globally, a low awns N accumulation was observed in "ICARDA20" regardless of N treatment.

4.4.4. Grain Protein Concentration

In order to evaluate the effects of salinity and N nutrition on grain quality, grain protein concentration was determined. The results showed that across the years the relation between GPC and GY was negatively correlated.

Salt stress significantly decreased GPC by 18%, in fact, the average GPC of the four genotypes was 9,2% and 7.5% under low and high salinity respectively. N supply enhanced significantly GPC in low saline condition (8.4%; 9%; 9.5% and 10% under 0N, 50N,100N and 150N respectively), while any amelioration was observed under salinity. In contrary, genotypic variability was observed only in salt stressed plants. In fact, "Souihli" and "Barley Medenine" allocated more N to grain (8% and 7.8% respectively) compared to "100/1B" and "ICARDA20" (7%). Based on the negative relationship between GY and GPC, it is not surprising that "Barley Medenine" -characterized by low yield- showed a high GPC but very interestingly for "Souihli"which performs high productivity.

4.5. Correlation between main traits

To describe the main important relationship resulting in GY, scatter plot was studied by compiling the three years data of each genotype grown under different levels of N treatments and both low and high saline conditions. Results showed that although that TKW is a main component determining GY, there was a moderate relationship between GY and TKW only for the tolerant "100/1B" ($r = 0.63$) and the improved "ICARDA20" ($r=0.4$) (Figure 6.A). Whereas GY was strongly correlated with grain number for all genotypes (Figure 6.B). In turn, grain number was directly correlated with N uptake (Figure 6.C), and N uptake was strongly correlated with biomass production (Figure 6.D). All genotypes showed a strong correlation with slight difference in correlation coefficient r.

GPC was subjected to clear variability between genotypes and saline treatment effect. For this reason, data of each genotype grown under different N rate at the three year were tested separately under low or high salinity. Through scatter plots we can understand if the obtained correlation was due to the saline conditions or genotypic differences (Figure 7).

Without salinity, GPC was moderately correlated with GY in all genotypes except "Souihli" which showed a strong correlation (Figure 7.A). Under salinity all genotypes showed a negative correlation between GY and GPC, only in "Souihli" this relation was positive ($r=0.64$) (Figure 7.B)

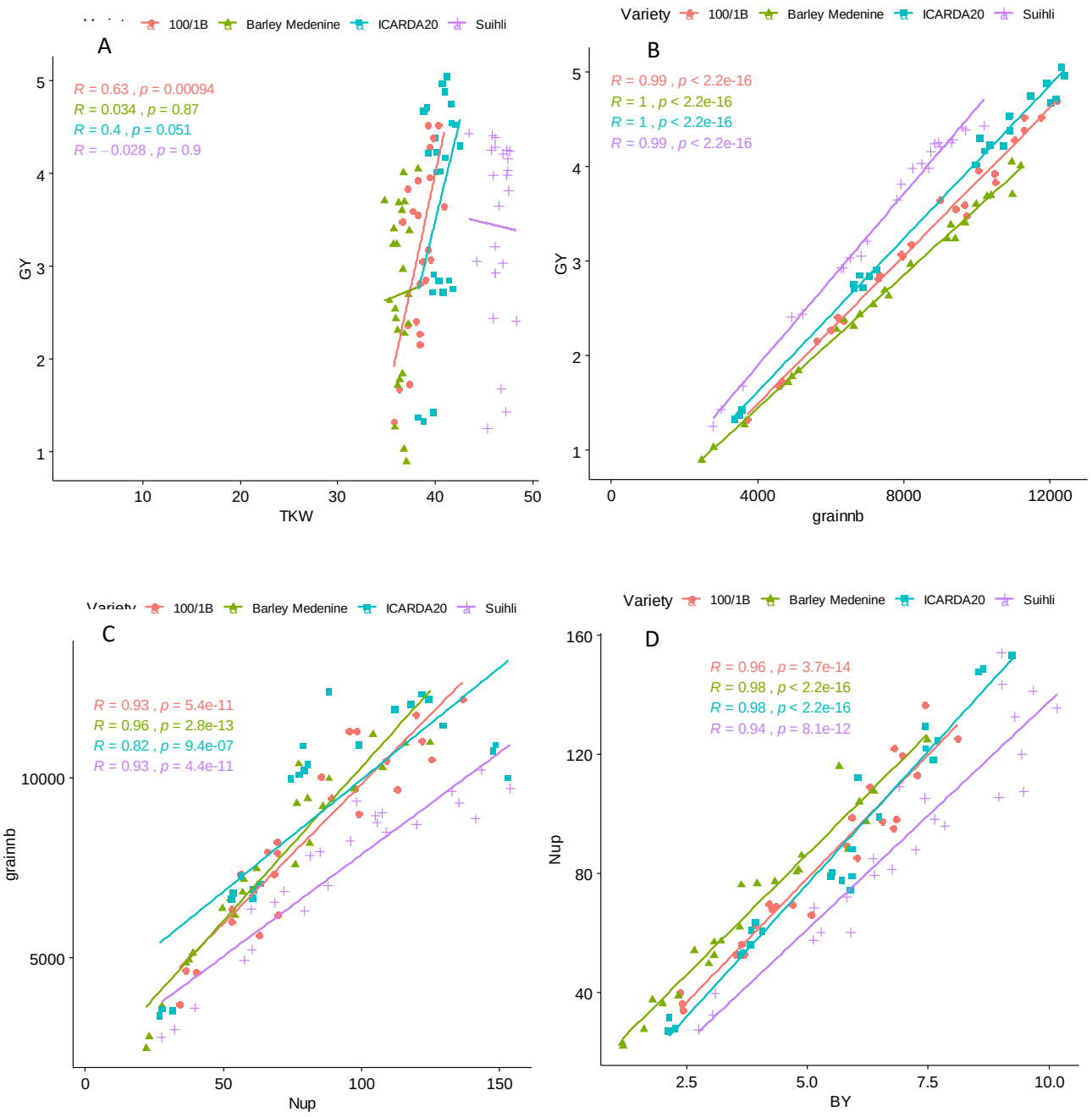

Figure 6: Main Relationship related to GY: A: relationship between grain yield (GY) and thousand kernel weight (TKW); B: relationship between grain yield (GY) and grain number (grainnb); C: relationship between grain number (grainnb) and N upatek (Nup); D: relationship between N upatek (Nup) and biomass yield (BY).

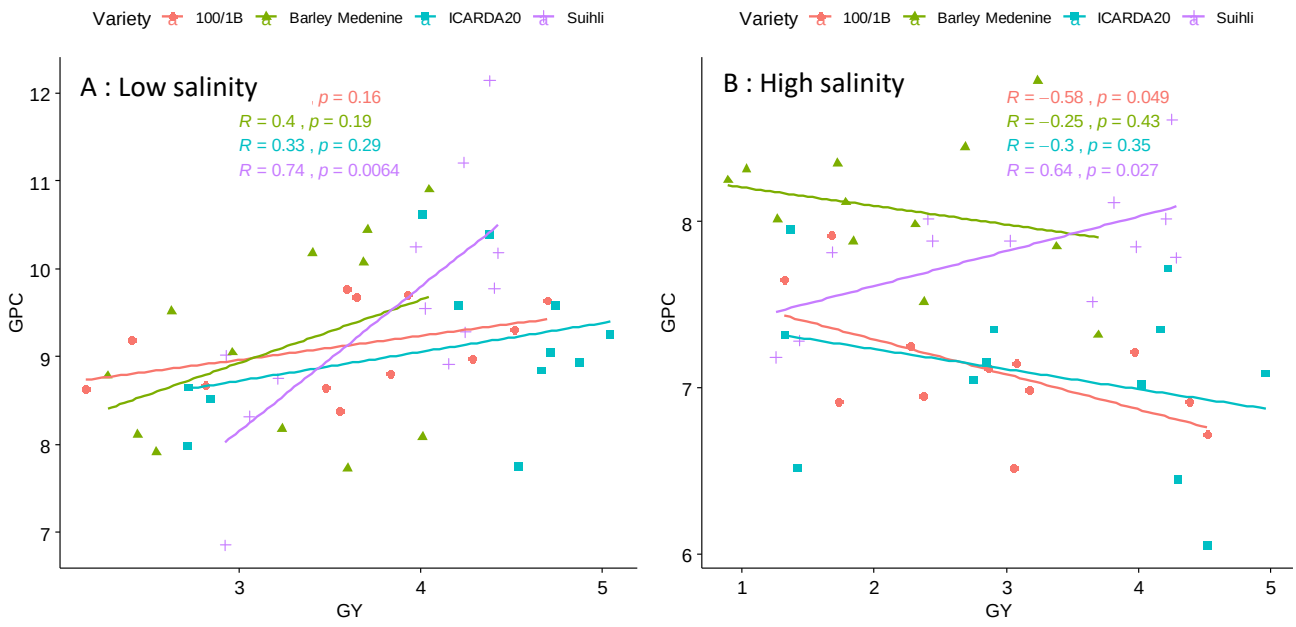

Figure 7: Relationship between GY and GPC under control (A) and saline (B) conditions.

4.6. Stepwise analyses of grain yield and N related traits

Based on the sharp relationship between N uptake and GY; traits related to N uptake portioning (N accumulation in straw, grain, awns) and GPC were used as independent variables to establish a multiple stepwise model explaining GY variations at both saline conditions. Regression analysis showed that grain N accumulation and GPC under low saline condition, and Grain N accumulation, straw N accumulation and GPC under salinity were selected by the model to be the most effective traits explaining GY.

The stepwise analysis revealed that 98,4% and 99,3% of GY variation under low saline and high saline conditions respectively was explained by a model which includes N metabolism traits (Table 5).

The model revealed that major GY variation was explained by GPC variability with a slope of -0.4 and -0.33 under low and high saline conditions respectively. Whereas, GY was positively proportional to grain N accumulation, with higher slope under salinity (0.085) compared with low saline condition (0.066). In other terms, as GPC increased (more N allocated to grain), GY was found to be decreased; while as grain N accumulation increased, GY tends to be enhanced. Straw N accumulation represents a minor role to predict GY variation only under salinity, with a slope of -0.012, which reflects the distribution of N remobilization to grain and its negative effect on GY.

Table 5: Multiple linear regressions analyses (stepwise) explaining the variation of grain yield (GY) in barley genotypes in each salinity level as a dependent variable. Parameters related to Total N uptake distribution (N accumulation in grain, staw and awns) with GPC were used as independent variables.

Levels of significance: ***P < 0.001; **<0.005

Dependet variable	Salinity	Variable chosen	Estimate	Standar Error	t	Significance	R2	Adjusted R2
GY	low salinity	Constant	3.696519	0.151737	24.36	0.00***	0,984	0,983
		Grain N content	0.065728	0.001379	47.67	0.00***		
		GPC	-0.424111	0.021687	-19.56	0.00***		
		Final model	**GY= 0,066 Grain N content - 0,42 GPC +3,7**					
GY	high salinity	Constant	2.534152	0.183764	13.790	0.00***	0,993	0,992
		GrainN content	0.085526	0.002495	34.279	0.00***		
		Straw N content	-0.012153	0.003958	-3.071	0.003**		
		GPC	-0.335561	0.023466	-14.300	0.00***		
		Final model	**GY= 0,085 grain N content - 0,012 straw N content- 0,33 GPC+2,5**					

5. Discussion

5.1. Grain yield and its component

Grain and biomass yield variability between years especially under salinity was the consequence of the differences in total amount of rainfall and its distribution. The positive correlation between productivity under salinity and precipitation suggests that rainfall water decreases soil salinity and enhance yield by leaching of salts from the root zone (Moreira Barradas et *al.*, 2015).

Our results suggest that the important rainfall amount in the second year, also its coinciding with stem elongation -which represents the most active developing period of crops (Seif and Pederson, 1978)-enhances markedly yield of plants irrigated by saline water. Similarly, wheat productivity in Mediterranean environments was influenced by annual rainfall and resulting in inter-annual variability (Dang et *al*., 2006); more precisely Austin et *al.* (1998) underlined the evident relation between grain yield and rainfall during stem elongation.

The magnitude of yield increase was less with a continuously increasing of N; at certain level of N supply depending on genotypes, grain yield reach a plateau and further N application decreased yield may due to nutrient toxicity or to the indirect effect such as lodging and disease or antagonisms with other elements (Godard et *al*., 2008). Our results showed that grain yield changes are due to the grain number variation. Comparable results have been reported in Mediterranean environment, where wheat grain yield increased with N application up to 100

kg N ha^{-1}, then grain number decreased with high N fertilization (Abad et *al*., 2004, wang et *al*., 2011, Chamekh et *al*., 2016). Grain yield difference between plants irrigated by normal and saline water in response to N is mainly due to the determining effect of salinity on total N uptake which was severely reduced probably because of the biomass production decrease. In fact, under salinity, the decrease in biomass production and N uptake which are strongly correlated with grain number, led to decline the number of grain per area, and consequently decrease grain productivity.

5.2. N uptake and distribution

N uptake and portioning in plants involves many growth and development aspects which needs to be considered in the regulation of N absorption and assimilation (Gastal and Lemaire, 2002). Correspondly with some other studies our data proved that salinity disrupt N remobilization and partitioning patterns (Ciampitti and Vyn, 2013; de Oliveira Silva et *al.*, 2017). N fertilizer alleviates the detrimental effect of salinity on total N uptake and N accumulation in grain, straw and awns. The greatest enhancement of total N uptake and its distribution caused by fertilizer was observed under salinity. This improvement is due to the further availability of mineral N in soil provided by ammo-nitrate (Gastaland Lemaire, 2002). Similar results has been shown in previous studies in wheat and rice (Guo et *al*.,2016; Wang et *al*., 2011; Barraclough et *al.*, 2010).

Interestingly, the tolerant "Souihli" reach its maximum grain and biomass yield under 100N, but continued to take up N fertilizer under 150N; these results hypothesized that luxury N accumulation not used for grain and biomass production but incorporated into "N reserve pool" could buffer against stresses or/and contributes to the improvement of grain qualities. The findings that "Souihli" efficiently absorb and allocate N to grain, together with the possession of the highest grain N accumulation, grain weight and grain yield which results in great grain protein yield support our hypothesis. In the same context, Plénet and Lemaire (2000), Ciampitti and Vyn (2012), and Nasielski et *al.* (2019) proposed that storage N pool could may be reduce the impact of N stress during grain filling.

The finding that only "Souihli" genotype showed a positive relationship between GY and GPC in both saline conditions reinforce the hypothesis that luxury N accumulation was used to enhance GPC. Lopez-Bellido et *al*., (2004) approved that at certain level of N supply both yield and GPC increase with increasing N fertilizer only when N uptake is high (Barraclough et *al*., 2010) which was proven in "Souihli". The negative correlation between GY and GPC in the rest of genotypes under salinity is mainly due to the requirement of N for both GY and grain quality (Barraclough et *al*. 2010, hawkesford, 2017). In fact, high productivity depend on

continuing photosynthesis in plant and therefore the maintain of a minimum concentration of catalytic N in leaf is required (Barraclough et *al*., 2010).

Our results showed that grain N accumulation largely depends on total N uptake as reported previously in wheat (Kichey et *al*., 2007). As N supply increased grain number, and in order to satisfy grain N demand plants increased N remobilization (Barraclough et *al.*, 2010).

N portioning between straw grain and awns was regulated via internal mechanism. Our results suggest that the excess of N is not remobilized to grain, it can remain in straw or allocated to awns depending on genotype. In fact the improved "ICARDA20" marked by its low requirement of N to achieve maximum yield accumulate the excessive amount of N in straw (42% of the total N uptake compared with 27% under sub 150N doses); whereas the landrace "100/1B" characterized by its sensibility to high N supply accumulate it in awns (13,5% of the total N uptake compared with 8.5% under sub 150N doses). In the same context, it has been reported that high N nutrition increased stomatal conductance, which in turn increased the loss of N from the top of plants (Harper et *al*. 1987; Farquhar et *al.* 1980). Our finding suggest that the high proportion of awns production to biomass and spike weight in "Barley Medenine" compared to other genotypes, evidently under salinity (data not shown) could explain the sensitivity of this genotype. In fact, awn development known to compete ovary growth for assimilate, and then influence grain yield because of the evident relation between grain size and number with ovary size (Guo et *al*., 2015; Xie et *al.*, 2015). Rebetzke et *al*. (2016) report that allocation of assimilates to developed awns reduce grain number and increase sterile spikelets number. The high N accumulation in "Barley Medenine" awns associated with the low grain N accumulation suggest the alteration of N allocation pattern to the spike. The influence of awns development on grain yield and assimilates redistribution within the spike has been previously reported by Guo and Schnurbusch (2016).

5.3. Grain quality or grain number?

Correspond to many other studies (Abda et *al*., 2004; Ottman et *al.*, 2000), our results showed that N fertilization improves grain quality of plants grown without salinity. N supply had no effect in grain quality under salinity which in turn decreases GPC; this effect is mainly due to the perturbation of N absorption in saline environment. The finding that both grain number and GPC increased in low saline condition while it is just grain number increased under salinity suggest that N allocation to sinks firstly improves grain number rather than protein content; only under plentiful N condition (low salinity) are both grain number and protein content maximum achieved **(**Zhang et *al.*, 2015; Perchilik and Tegeder, 2017). Then, the response of

barley genotypes to N fertilization was more evident for the productivity than for the grain quality, giving an opposite type of response to that of wheat (Abad et *al*., 2004).

6. Conclusion

Our works shows the suitability of the Mediterranean arid climate for barley production by optimal N fertilization alleviating the negative effect of salinity. Maintaining an efficient N uptake under stressful circumstances is one of the keys to obtain high grain yield and quality. N uptake and distribution can be a precious tool to predict the tolerance or the susceptibility of Barley genotypes. Luxury accumulation of the excessive N and its incorporation into "N reserve pool" is a researched phenomenon enhances salt tolerance and contributes to a high grain quality. In addition, it can be a used tool to select the most tolerant genotypes face to salinity. Further physiological and molecular studies are required to better understand luxury N reserve mechanism.

Chapter II: Nitrogen use efficiency (NUE) components in barley genotypes (*Hordeum vulgare* L.) contrasting in salinity tolerance grown in Mediterranean arid environment

This chapter is prepared for submission in journal of

Cereal Research Communications

1. Abstract

Nitrogen losses reduce crop yields and impose adverse effects on soil and environment. Unraveling the difficulty underpinning nitrogen use efficiency (NUE) in different genotypes can be approached via studying Nitrogen uptake efficiency (NupE) and Nitrogen utilization efficiency (NutE). The main objective of this research is to evaluate NUE in four contrasting barley genotypes and evaluate physiological internal mechanisms related to yield and N dynamics. Four Barley genotypes ("100/1B" and "Souihli" are tolerant to salinity, while "Barley medenine" and "ICARDA20" are susceptible) grown for 3 cropping seasons under 8 combinations of N (0, 50, 100, and 150 kg N/ha) and irrigation water salinity (1.8 and 9.2 dS/m) in arid climate. Under salinity, NUpE decreased when rainfall is limited, whereas without salinity it decreased when the rainfall is important. Saline irrigation decreased the efficiency to uptake N by 40.3% and NUE by 28.5% but increased NUtE by 18.6%. NUpE decreased with increasing N rate: 9%, 16% and 27% were the reduction caused by 50, 100 and 150 KgN/ha respectively. The tolerant "Souihli" showed the highest NUpE (65.3%) while the improved "ICARDA20" was the most efficient in using N (45.75 kg grain/kg uptaken N) which resulted in higher NUE for these two genotypes (25.5 Kg/KgN). N concentration in straw, grain and awns increased with increasing N supply. Interestingly "Souihli" allocate more N to grains and less N to straw and chaff. In order to evaluate the relationship between GY and NUE components, correlation analysis was established, and results showed that under salinity, 45% and 33% of the variation in grain yield was explained only by NutE in "ICARDA20" and "Souihli" respectively.

Key words: salinity, barley, NupE, NutE, NUE.

2. Introduction

Barley is a worldwide major crop grown in around 70 million hectares (USDA, 2020) mainly in arid and semi-arid areas (Akar et *al*., 2004). Barley is the fourth most important worldwide cereal crop after wheat, maize, and rice (FAO, 2017). The grains are essentially used as animal feed, malt, and human food resources. Generally, barley production is more resilient to climate change, seasonal variation, salinity and low soil fertility. In arid areas, barley is more productive than wheat and other small grains (Hammami et *al*., 2017). In addition, barley plays a key role as a model plant allowing advances in genetics, cytogenetics, physiology, pathology, biochemistry, and biotechnology studies in plants (Harwood, 2019).

Nitrogen (N) fertilization is a key agronomic practice for optimal crop quality and maximum yield. The availability and the uptake of N was closely related to gain and biomass yield (Barraclough et *al*,. 2010). The introduction of N-responsive varieties was among the causes of excessive fertilizers use. Intensive use of N fertilizers leads to the accumulation of nitrate, which can leach down to the soil layer and thus negatively affect the environment (Rasool et *al*., 2018). Nitrate leaching should not be underestimated because of its negative effects on the ecosystem, global climate, and human health (Coskun et *al*., 2017). Therefore, excess amount of N can induce lodging in barley and reduce yield (Anbessa and Juskiw, 2012). Nitrogen use efficiency (NUE) for cereals was estimated on average to just 33% which represents a major N fertilizer inefficiency (Hawkesford et *al*., 2017). The challenge is to improve crop productivity and quality in arid regions characterized by soil and water salinity, minimize production cost and environmental pollution through the use of genotypes with high NUE under salinity. A crop with optimal N use would not only take up N from the soil efficiently, but would also utilize the absorbed N more efficiently for production (Burstin et *al*., 2007). The optimization of N input and the improvement of genotypes with optimal NUE is among the main targets of research on plant nutrition (Song et *al*., 2019). Whereas, in general, studies focus on the improvement of NUE under specific N treatments and rarely address the environmental effect on the different NUE component (NUtE and NUpE) (Perchilik and Tegeder, 2017). Knowledge about regulation of N uptake and utilization in saline arid region has remained limited. In fact, investigations on salinity and N nutrition have been conducted under controlled conditions; while plant response to salinity-nitrogen interaction in field can be affected by several factors such as soil characteristics, soil initial N, cultivars, organic matter and availability of other nutrients. Studying the relationship between salinity and N metabolism in natural environment is very complex and challenging objective. In this context, the present study was conducted in field to evaluate and deeply understand NUE and its components in four contrasting barley genotypes under different combinations of N supply and water salinity. We

hypothesized that NUE contribute to salinity tolerance, and that NUE components largely affect plant growth and productivity under salinity via the regulation of source-sink connection.

3. Materials and methods

3.1. Field site and climates conditions

Field experiment was performed at the arid region El Fjé-Medenine (33°26′54"N,10°56′31"E) situated in the South East of Tunisia (location of the study area is shown in the Figure 1 of the chapter I). Field experiment was conducted for three years (Y1: 2016–2017, Y2: 2017–2018 and Y3: 2018-2019). A meteorological station was installed in the experimental site to properly characterize climatic conditions. The quantity and distribution of precipitation, maximum and minimum temperatures were presented in the Figure 2 of the chapter I). Data of temperature are comparable across the three cropping seasons, whereas the rainfall amount was notably different particularly for Y2 distinguished by an important rainfall (290 mm). Y1 is characterized by low rainfall of 156 mm and Y3 is caracterized by an intermediate precipitation (167 mm).

3.2. Soil characteristics

The soil of the experimental field is classified as sandy soil composed by 4.5 % clay, 14.8% silt, and 80.7% sand. It is characterized by a low organic matter content (0.9%) and high electrical conductivity (EC= 2,8 mS/cm) and pH=7,7. The content of active limestone and assimilable phosphorus were low in the soil: an average of 5% and 9 ppm. Apparent soil density is around 1.2. Initial content of nitrate and ammonium in the soil for the three years is summarized in Table 1.

Table1: Nitrate and ammonium content in the soil.

	Initial N-NO_3^- content (kg N ha^{-1})	**Initial N-NH_4^+ content (kg N ha^{-1})**
2016-2017 (Y1)	**44.5**	**33.5**
2017-2018 (Y2)	**35**	**57**
2018-2019 (Y3)	**20**	**38**

3.3. Plant material and experimental design

During the three cropping seasons, four barley genotypes with contrasting aptitude to salinity were cultivated on field. The "100/1B" and "Souihli" are considered as tolerant, while "Barley medenine" and "ICARDA20" as susceptible (Hammami et *al.*, 2017; Table 2). Genotypes seeds were sown by hand at a density of 200 seeds m^{-2}. A split-split plot design was adopted. Experimental field was divided into two sub-plots, and three blocks were planned perpendicularly to the sub-plots. Two water salinity levels were used for irrigation in each block: low (1.8 dS/m) and high (9,2 dS/m) saline water. Every plot consisted of 6 rows of 2 m length spaced by 20 cm.

Table 2: characteristic of tested Barley genotypes (Hammami et *al*., 2017)

Genotype name	Tolerance to salinity	Type	Location	Soil salinity in the origin area (dS m^{-1})*
"100/1B"	Tolerant	Landrace	Oman	2
"Souihli" (beldi/sahli)	Tolerant	Landrace	Tunisia:Mahdia,ksas	2.7
"Barley Medenine"	Susceptible	Landrace	ICARDA/Tunisia	1.3
"ICARDA20"	Susceptible	Cultivar	ICARDA	0.3

*Soil salinity (in test performed using Electrical Conductivity 1:5 ("EC one-to-five")

3.4. Nitrogen and Irrigation Management

Four N fertilizer levels were applied: 0, 50, 100, and 150 kg N/ha (0N, 50N, 100N and 150N) at three barley growth stages: 30 % at early tillering (Z13), 40% at stem elongation (Z16) and 30% at the second node stage (Z32). N fertilizer was added as ammonium nitrate form (33,5%). Plants were irrigated with low saline water as control (1.8 dS/m) and high saline water (9,2 dS/m). Irrigation was carried out through a drip system. The barley growth water needs were provided according to climatic and soil data.

3.5. Sampling and data measurements

The four central rows were harvested at maturity; plants were separated into awns, straw and seeds. Grains were collected using a laboratory thresher (Wentersteiger, LD-180, Germany). After drying at 65° for 48h, all samples were weighted and ground by a rotor mill to obtain a fine powder used for N analysis. Samples were digested according to Kjeldahl procedure, and N concentration was determined using Cataldo et *al*., (1974) method. Before sowing and after harvest soil mineral N was determined. Soil samples were collected to a depth of 0.6 m at intervals of 0.2 m. Nitrate content was measured using Devarda's Alloy reagent method (Sims et *al*., 1995) and ammonium was calculated according to the distillation titration proceeding method (Rhine et *al.*, 1998).

Based on the total N content of soil and plant, Nitrogen use efficiency (NUE) components was calculated according to Good et *al.*, (2004):

1) N uptake efficiency (NUpE): Total N uptake/crop N supply (fertilizer N + soil mineral N at sowing) × 100 (%)

2) N utilization efficiency (NUtE): grain yield/total N uptake (kg/kg N)

3) N use efficiency for grain yield (NUE): grain yield/crop N supply (fertilizer N + soil mineral N at planting) (kg grain yield/kg N; %)

4) N Harvest index (NHI): N contents in grains/ plant N content (Barraclough et *al.*, 2010)

3.6. Statistical analyses

Data were analysed using R software (R-64 3.6.1). To evaluate the effect of salinity, Nitrogen treatment and genotypes, ANOVA analysis was established. The significance of factor was performed according to p-values. Duncan test ($p < 0.05$) was used for means comparison test. Linear regression analysis was conducted to identify the traits related to NUE components and grain yield. To design figures GraphPad Prism 8 program was used.

4. Results

4.1. Nitrogen efficiency components

1.1.2. 4.1.1. N uptake efficiency (NUpE)

Nitrogen uptake efficiency in grains was significantly affected by year (Y), salinity (S), genotypes (G) and N fertilization and the interactions Y × G , Y × S and N × S (Table 3).

Table 3: Analysis of variance showing mean square of different trait for the 4 genotypes under different N and saline treatments. *: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$

	Df	N up E	N ut E	NUE	NHI	Grain [N]	Straw [N]	Chaff [N]
Year (Y)	1	10023***	22	2943***	0.3276***	4.719***	1.6664***	4.787***
Genotype (G)	3	3692***	534***	621***	0.0331***	0.206***	0.1381***	0.285***
Salinity (S)	1	61381***	5285***	4372***	0.0067	6.240***	0.3926***	2.389***
N level (N)	3	4175***	262*	1301***	0.0173*	0.210***	0.0914***	0.373***
Y *G	3	891*	15	213**	0.0150*	0.054	0.0297**	0.173***
Y *S	1	1825**	2533***	196*	0.0259*	1.577***	0.4291***	0.839***
G*S	3	208	183.	56	0.0112.	0.124*	0.0260*	0.154***
Y*N	3	264	134	3	0.0224**	0.242***	0.0151	0.160***
G*N	9	351	39	74.	0.0040	0.068*	0.0179.	0.026***
S*N	3	2409***	677***	1076***	0.0157*	0.317***	0.0287*	0.330***
Y*G*S	3	347	59	34	0.0372***	0.037	0.0354**	0.170***
Y*G*N	9	327	25	52	0.0069	0.028	0.0304***	0.059***
Y*S*N	3	125	8	69	0.0014	0.071.	0.0255*	0.292***
G*S*N	9	256	74	67	0.0110*	0.030	0.0285**	0.043***
Y*G*S*N	9	723**	13	101*	0.0043	0.038	0.0135	0.031***
Residuals	224	249	72	41	0.0047	0.035	0.0094	0.003

The third year (Y3) characterized by an intermediate rainfall amount was distinguished by the highest NUpE. The interaction Y × S showed that under salinity, NUpE were more sensitive to rainfall deficiency (Y1), whereas without salinity it is more sensitive to the high rainfall amount (Y2) (Table 4).

Salinity affected negatively NUpE and caused a decrease of 40.3%. NUpE decreased with increasing N rate: 9%, 16% and 27% were the reduction caused by 50N,100N and 150N respectively compared to the non-fertilized plants (Figure 1). In fact, for all genotypes the highest

NUpE was observed in unfertilized plot except the improved "ICARDA20" which showed its maximum uptake potential under 50N.

The highest NUpE was observed in "Souihli" (65.3%) and the lowest value in "Barley Medenine" (48%), whereas a comparable value was observed in "100/1B" and "ICARDA20" (59%).

Table 4: Means of Nitrogen uptake efficiency (NUpE) at three years under low and high saline irrigation.

	Year	NUpE
Low salinity	2017	72.19289 [b]
	2018	64.69142 [c]
	2019	80.47716 [a]
High salinity	2017	33.00755 [f]
	2018	43.13568 [e]
	2019	53.62443 [d]

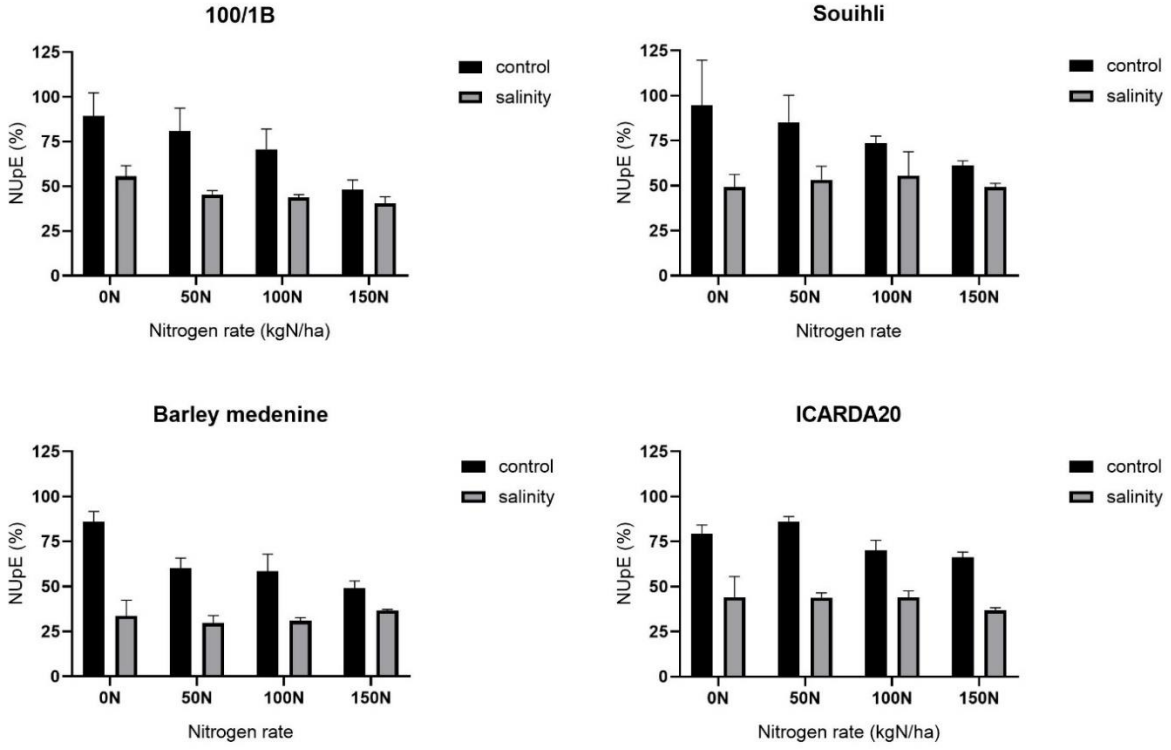

Figure 1: Nitrogen uptake efficiency (NUpE) in different genotypes grown under 0N, 50N, 100N and 150N treatment under control (black) and saline irrigation (grey).

1.1.3. 4.1.2. N utilization efficiency (NUtE)

NUtE was significantly affected by salinity (S), N fertilization rates (N), genotypes (G), and the interaction Y × S and N × S (Table 3).

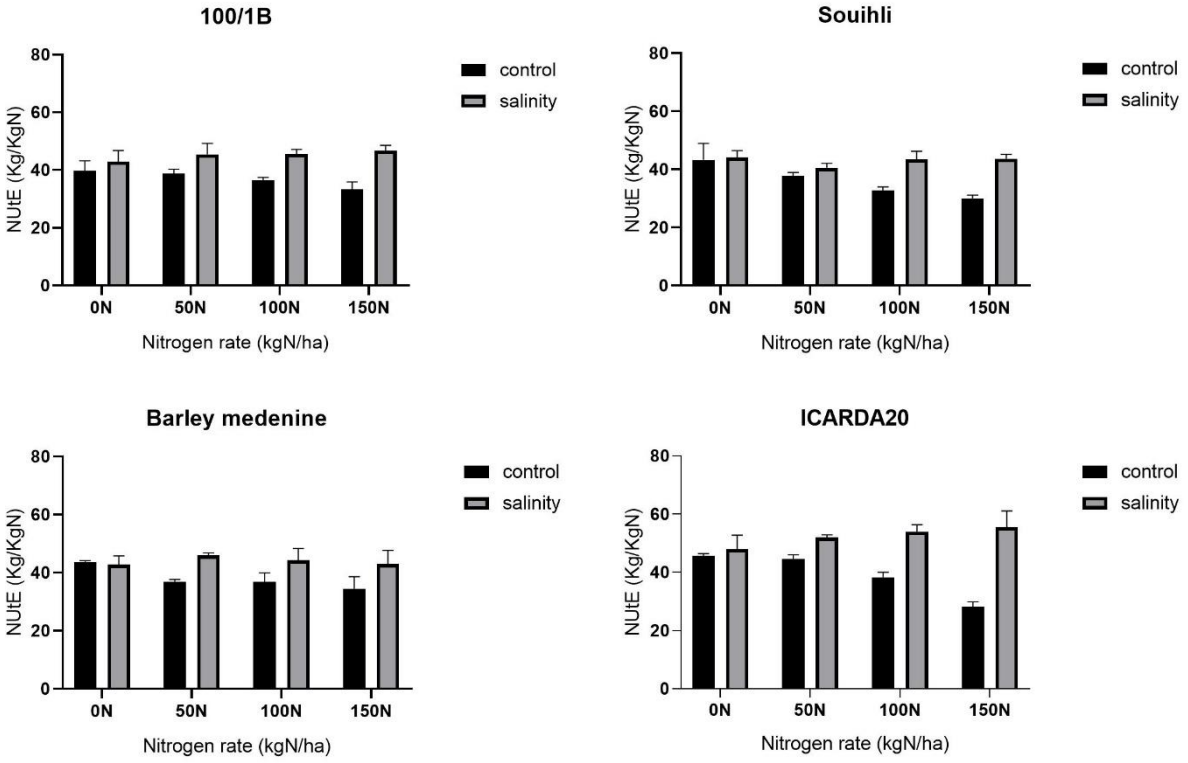

Figure 2: Nitrogen utilization efficiency (NUtE) in different genotypes grown under 0N, 50N, 100N and 150N treatment under control (black) and saline irrigation (grey).

NUtE increased by 18.6% under water salinity for all tested genotypes. There was no clear change in NUtE with the increase of N rate. In fact, NUtE decreased slightly (7.8%) only under high N fertilization (150N) (Figure 2).

Results showed a genotypic variation in NUtE. In fact, "ICARDA20" was the most efficient in using N with an average of 45.75 kg grain/kg N uptake compared to 40.5 Kg grain/Kg N in the other genotypes.

4.2. Nitrogen Use Efficiency (NUE)

NUE decreased with increasing N supply by 10%, 20%, 35.6% under 50N, 100N and 150N, respectively (Figure 3). In addition, NUE has been reduced by 28.5% under salinity. The Improved genotype "ICARDA20" and "Souihli" landrace showed the maximum NUE (25.5 Kg/KgN), while the sensitive "Medenine" was the less efficient genotype (19.2 Kg/KgN). "100/1B" displayed an average NUE of 23,3 Kg/KgN. Interestingly, the landrace "100/1B" showed a relative NUE stability under different treatments compared with other genotypes.

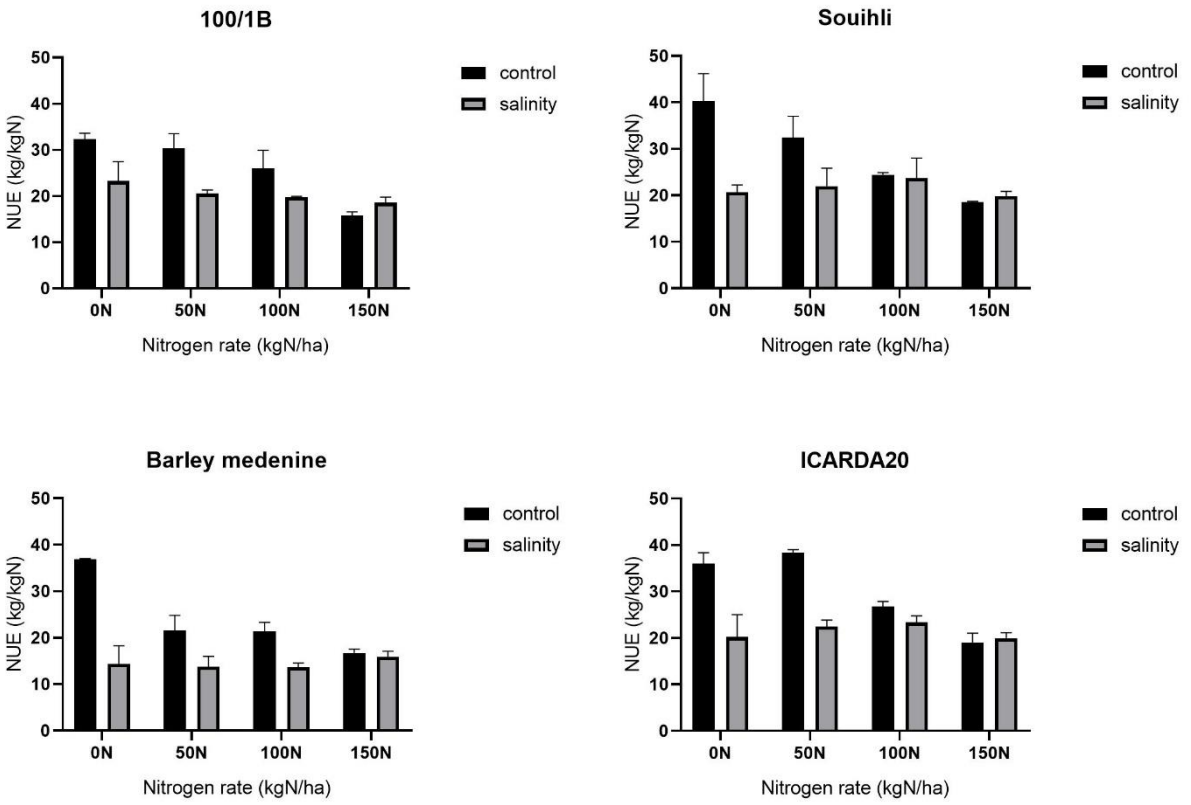

Figure 3: Nitrogen use efficiency (NUE) in different genotypes grown under 0N, 50N, 100N and 150N treatment under control (black) and saline irrigation (grey).

4.3. Nitrogen Harvest Index (NHI)

Nitrogen harvest index ranged from 0.5 to 0.65 depending on the interaction between G × N × S (Table 3). The average of NHI values for the 3 years were 0.62, 0.59 and 0.54 in 2017, 2018, 2019, respectively. Globally, significant genotypic differences in NHI value were recorded with 0.61 for "ICARDA20" and 0.59 for "Barley medenine" and 0.57 for "Souihli" and "100/1B" (Table 5). Results showed that there was no variation in NHI caused by salinity. In addition, there was no noticeable trend in NHI with N-level; NHI was the least affected variables by N-rate. Average NHI values were 0.59 at 0N, 50N and 100N, and 0.56 at 150N.

4.4. Nitrogen concentration

N in grain, straw and awns were determined to evaluate the effects of salinity, N nutrition and genotypes. A significant reduction of 13%, 18% and 23% were observed under saline irrigation in straw, grain and awns respectively (Table 5). N in straw and grain were increased in all genotypes under high N application, unchanged under moderate N (50 and 100N), and decreased without N

fertilization. The same results were observed in awns except for the significant difference between 50N and 100N. "Barley Medenine" showed the highest N in straw, grain and chaff compared to other genotypes. Interestingly, results showed that "Souihli" allocate more N to grains and less N to straw and awns (Table 5).

Table 5: Genotype and treatment effects on N concentration in straw, grain and chaff.

	Straw [N]	Grain [N]	Awns [N]	NHI
Genotype				
"100/1B"	0,55^{a}	1,42^{b}	0,73^{b}	0,56^{c}
"Souihli"	0,48^{b}	1,52^{a}	0,64^{d}	0,57^{ab}
"Barley Medenine"	0,57^{a}	1,50^{a}	0,78^{a}	0,59^{ab}
"ICARDA20"	0,49^{b}	1,41^{b}	0,67^{c}	0,61^{a}
Salinity				
low salinity	0,56^{a}	1,61^{a}	0,80^{a}	0,59^{a}
high salinity	0,48^{b}	1,32^{b}	0,61^{b}	0,58^{a}
Nitrogen				
0N	0,48^{c}	1,40^{c}	0,62^{d}	0,60^{a}
50N	0,50^{bc}	1,45^{bc}	0,69^{c}	0.6^{a}
100N	0,53^{b}	1,48^{ab}	0,73^{b}	0.59^{a}
150N	0,56^{a}	1,53^{a}	0,79^{a}	0.56

4.5. Correlations

To evaluate the relationship between GY and NUE components, correlation analysis for each genotype under both saline conditions was realized. Results showed that NUtE was positively correlated with GY only in the landrace "100/1B" and the improved "ICARDA20" grown under salinity. The equation explaining GY variations in terms of NutE in all genotypes was determined at both saline conditions (Table 6).

Table 6: linear regression analyses explaining the variation of grain yield (GY) in terms of NutE in barley genotypes in both salinity level.

	Genotype	R^2	r	Equation
Low salinity	"100/1B"	0,136	-0,37	Y= -1,567x+42,481
	"Souihli"	0,615	-0,78	Y=-7,5355x+64,851
	"Barley Medenine"	0,431	-0,66	Y= -0,0979x+6,9287
	"ICARDA20"	0,077	-0,28	Y=-2,3767x+48,959
High salinity	"100/1B"	0,335	0,58	**Y=1,6257x+40,484**
	"Souihli"	0,000	-0,012	Y=-0,0249x+43,008
	"Barley Medenine"	0,000	0,0015	Y=0,052x+43,89
	"ICARDA20"	0,457	0,68	**Y=2,2777x+44,976**

Results revealed that 33% and 45% of GY variation under salinity was only explained by NutE in "100/1B" and "ICARDA20" respectively.

The equation revealed that under salinity, GY variation was explained by NutE variability with a slope of 1.6 and 2.3 in "100/1B" and "ICARDA20" respectively. As showed in the table 6, as NutE increased, GY tends to be improved. Interestingly the improved "ICARDA20" showed a higher slope compared with "100/1B".

5. Discussion

Adopting an optimal fertilizer management or choosing an efficient genotype are two ways to improve NUE in barley. NUE can be enhanced by coordinating N applications to crop requirement and weather. Weather has a major effect on the availability and the accessibility to N fertilizer, which influence cereal growth, and yield (Barraclough et *al.*, 2010). The difficulty of predicting weather and the major influence of rainfall in arid climate like in southern Tunisia is the main barrier to N fertilizer management. The second approach to improve NUE in cereal is to breed genotypes able to efficiently absorb N from soils (NUpE) and use it to produce yield (NUtE).

The regulation of N uptake and utilization is multifaceted, and may include N metabolite signaling mechanisms in plant shoot and root as well as shoot-to-root communication (Perchilik and Tegeder, 2017). Our study showed that the improvement of NUE is related to a larger N uptake or net N remobilization from the storage organ. In fact, based on the genetic variation in NUpE and NUtE, our data showed that the most efficient genotypes were the tolerant "Souihli" characterized by its high uptake ability and the improved "ICARDA20" recognized by its high N remobilization.

The observed NUpE variation through year can be explained by the rainfall amount. In fact, under non-saline condition, NUpE decreased when rainfall increased which can be explained by N loss through nitrate leaching or denitrification. In the same context, Sharma et *al*., (2017) reported that precipitation amount largely affect the availability of N to plants and the rate of organic matter mineralization. In addition, the sandy soil texture in our field study is probably a main factor enabling N denitrification losses. Whereas, under salinity, NUpE was increased when the amount of rainfall is important, which can be explained by the action of rainfall water in the decrease of water and soil salinity, and the mitigation of the disturbing effect on N uptake.

During N uptake, several loss processes can mainly affect nitrate, especially that ammonium nitrate is the main used fertilizer in Tunisia. Applying N in three stage (30 % at early tillering (Z13), 40% at stem elongation (Z16) and 30% at the second node stage (Z32)) during the vegetative growth (period of High N requirement) was our approach to minimize N loss and maximize crop recovery. Interestingly, the efficient capture of N from the soil was observed in the tolerant genotypes

("Souihli" and "100/1B"). Based on the genetic variation in NUpE between the tolerant ("100/1B" and "Souihli") and the sensitive genotypes ("ICARDA20" and "Barley Medenine"), the present study suggest that NUpE can be a determinant factor of genotype tolerance to salinity.

The N uptake decrease under salinity can be explained by the physiological alterations as leaf senescence process caused by salt ions, which affect N uptake and lead to "sink-limitation" and yield reduction (Ciampitti et *al*., 2016). Furthermore, NUpE reduction is probably explained by the antagonistic effect between salts ions (Na^+ and Cl^-) and NO_3^- and NH_4^+ provided by ammonium nitrate and/or the reduction of water absorption due to the osmotic changes in the root zone (Ashraf et *al*., 2018). Equally, this antagonistic relation can explain the decrease in N concentration in all parts of barley plants under salinity.

Results showed that the high N remobilization is the main breeding trait for improving NUE under salinity. NutE can be defined as the physiological efficiency of plant related to the N utilization for yield production (Ciampitti et *al*., 2016). Thus, to enhance NutE either (i) grain yield must be raised at fixed N-uptake, or (ii) grain yield must be sustained with less N uptake (Barraclough et *al*., 2010). Our results revealed that NUtE decreased by 7.8% under high N fertilization (150KgN/ha). In this context, Cox et *al*. (1986) revealed that high N fertilization before flowering led to increase N uptake and decrease N remobilization through making it less necessary (Barbottin et *al*., 2005). In addition, Perchilik and Tegeder (2017) revealed that when soil N is plentiful, the enhancement of N utilization efficiency is not highly required, and the allocation of the up taken N is sufficient to accommodate the sink N demand. Therefore, the increase of NUtE by 19% under salinity can be an internal equilibrium mechanism to compensate the decrease of NUpE caused by salinity. These results are in line with previous studies showing that NUtE influences NUE under N deficiency (Chardon et *al*., 2010; Mu et *al*., 2015).

Interestingly, "ICARDA20" showed the highest NutE and the strongest correlation between NutE and grain yield (45% of grain yield variation was explained only by NutE). Our results proved that N allocation from source to sink in the improved "ICARDA 20" is higher than the landraces; which suggests that the improved genotypes are better equipped in remobilizing N compared with landraces. Our data suggest that NUE was important in the N-starved barley plants due to more efficient N uptake (the total up taken N relative to the soil N), but NUtE was not changed. Whereas, despite the decrease of NupE under salinity, NUE was maintained due to more efficient N utilization for grain production. These findings supports that source-to-sink N transport manipulation can be an effective strategy to improve NUE and plant productivity under salinity.

When grown under high N all genotypes displayed significantly higher N in different plant parts, supporting the increase of root N acquisition and root-to different plant part allocation.

N transfer from stems and leaves would allow an increase in NHI and grain yield. Increasing NHI (grain yield*N%/Total N uptake) through the enhancement of grain yield or grain N concentration is an evident challenge because of the 'selfdestruct' hypothesis of Sinclair and De Wit (1975). In fact, when N root absorption is stopped and N is needed for grain protein, N is remobilized from leaves and stems which reduces photosynthesis (dependent on catalytic leaf N as Rubisco) and consequently decrease yield (Barraclough et *al.*, 2010). In this respect, grain yield and N concentration and accumulation in different plant parts are related. The high N concentration in the sensitive "Barley Medenine" in all plant parts (straw, grain and awns) can be explained by the low "dilution" effect as results of the low biomass and grain yield (chapter 1). In that case, N uptake was not organized into proteins and the level of N in plant tissue increase.

In conclusion, our work shows that the most efficient genotypes were the tolerant "Souihli" and the improved "ICARDA 20" because of their efficiency in uptake or using N. They could constitute a gene source for breeders to improve barley NUE. Our study suggest that NUpE can be a suitable index of genotype tolerance to salinity, and NutE could be an internal equilibrium mechanism to maintain an efficient NUE under stressful circumstances. Therefore, this work support that engineering N allocation from source to sink could be an effective strategy to improve NUE and plant productivity in a range of stressful environments. New understandings of differential expression of transcripts linked to N metabolism may provide new approaches and ways for improving NUE under salinity. From a biochemical point of view, a deeper understanding of the NUE mechanism would not only help to improve yield through superior nutrient performance but will also be related to the regulation of leaf senescence through the interplay of the C/N balance (Ciampitti et *al*., 2016). An integrated and multidisciplinary research on NUE and its processes is therefore required to fully understand the implications on barley grown under salinity.

Chapter III: Interactive effects of nitrogen nutrition and salinity on physiological responses in barley (*Hordeum vulgare* L.)

Accepted for publication in Annales de l'INRAT

1. Abstract

Understanding the interactive effects between salinity and nitrogen (N) fertilization is of great agronomic and economic interest to enhance barley growth and productivity. The objective of this work was to investigate whether N supply could alleviate the inhibitory effect of salinity on physiological and growth traits. Four contrasting genotypes ("100/1B" and "Souihli" are salt-tolerant genotypes while "ICARDA20" and "Barley Medenine" are susceptible) were grown under low (1mM KNO_3) and high (10 Mm KNO_3) N treatments in non-saline and saline (150 mM NaCl) conditions. Our results showed that N supply can alleviate the adverse effects of salinity and improve plant growth through the stabilization of the photosynthetic rate under salt stress and the attenuation of: (i) the increase of Na^+ concentration and uptake, (ii) the reduction of N uptake (iii) the decrease in biomass, height and tillers number, in salt stressed plants. Our findings support the hypothesis that Nitrogen use efficiency (NUE), sodium (Na^+) concentration, and potassium (K^+) uptake can be a useful index of barley tolerance against salinity. In fact, the tolerant landrace "100/1B" showed the highest NUE, K^+ uptake and the lowest Na^+ concentration. Whereas, the lowest NUE and K^+ uptake, and the highest Na^+ concentration were observed in the sensitive "Barley Medenine".

Keys words: Salinity, Nitrogen, Barley genotypes, NUE, K^+ uptake, Na^+ concentration

2. Introduction

Salinity is a worldwide problem that threatens to agriculture and plant production (Munns and Tester, 2008) especially in arid and semi-arid regions characterized by the shortage of water beside its poor quality, and the degradation of soil (Geissler et *al*., 2010 ; Munns and Tester, 2008). More than 20% of cultivated areas in the world are damaged by salinity (Shrivastava and Kumar, 2015). Hence, the need to develop species adapted to salinity to maintain sustainable agriculture and crop productivity in the affected region. Barley (*Hordeum Vulgare* L.) considered as a tolerant plant to salinity replacing wheat in these regions (Johnson and Flower, 1992). It is particularly a basic component of crop rotations and plays a crucial role in the combined crop-livestock production systems by ensuring food and feed source (Hammami et *al.*, 2017). Salinity imposes ionic imbalance in plants because of the high levels of chloride (Cl^-) and sodium (Na^+) ions in saline soil and brackish water (Ashraf et *al.*, 2018). Cl^- is known by its competition with Nitrogen nitrate form (NO^{3-}) (Abdelgadir et *al.*, 2005), while Na^+ shows an antagonistic effect with the ammonium form (NH_4^+) (Dluzniewskaet *al*., 2007). Thus, nutritive value, growth, and production of plants are affected by the increasing salinity (Farooq et *al*., 2015; Munns and Tester 2008). Distinct behaviors may be observed in plants exposed to salinity, depending on many factor: plant species and genotypes, tolerance and susceptibility of genotypes, the severity and the period of salt stress, type of salts, plant development stage (Yousfi et *al*., 2012, Ashraf et *al*., 2018). To avoid most of the damages caused by salinity, plants have developed a number of mechanisms, generally referred as salt tolerance, showing the property of a multigenic trait able to preserve plant development and metabolic functions (Cardi et *al.*, 2015). Alternative culture approaches to reduce the detrimental effects of salinity on plant growth and production represent a great interest for breeders. Convenient application of nitrogen (N) fertilizer is an effective practice to alleviate the adverse effect of salinity and improve production and nutritive value of plants (Chen et *al*., 2010). N has also been reported to improve the salinity tolerance of plants (Chen et *al*., 2010), because it can play nutritional and osmotic roles under saline conditions (Song et *al*., 2019). Considerable works are available regarding the alleviation of the negative effect of salinity by a proper application of N fertilizer in some crops such as, maize (Zea mays L.), rice (Oryza. sativa L.), wheat (Triticum aestivum L.), cotton (Gossypium spp.), and oat (Avena sativa L.) (Song et *al*., 2019). However scanty studies are available regarding the possible role of N fertilizer to alleviate the detrimental effects of saline stress in barley plants. Because of the genotypic variability it is crucial to select the tolerant genotypes adapted to saline stress and understand its defense mechanisms.

This study aimed to understand the interactive effects between salinity and N application on physiological and growth traits in four contrasting barley genotypes; and investigate how N fertilizer could alleviate the detrimental effects of salinity.

3. Materials and methods

3.1.Plant material and experimental design

To study plant response to salinity and N supply, a semi-hydroponic culture technique was conducted under controlled greenhouse conditions. Based on their contrasting response to salinity, four barley genotypes were chosen for this experiment: "Souihli" and "100/1B" are tolerant while "Barley medenine" and "ICARDA20" are susceptible (Hammami et *al.*, 2017; Ben Azaiez et *al.*, 2020). Barley seeds were sterilized with 3% sodium hypochlorite for 5 min, and then rinsed with deionized water. They were sown in 10L pot containing 70% perlite and 30% sand. A randomized complete block design with three replications (blocks) was adopted; in each block all genotypes, saline and nitrogen treatments were randomly distributed. During 2 weeks plants were irrigated with deionized water, and then a modified half strength Hoagland solution was used for irrigation. 4-weeks-old seedling N was supplied in the form of KNO_3 under two nutritional treatments (low N=1 and high N=10 mM KNO_3). Salt treatments (0 and 150 mM NaCl) started 5 weeks after sowing and were added incrementally: increasing by 50 mM a 3 day to reach the concentration of 150 mM. Nutrient solution was daily controlled for pH and plants were watered with 250 ml once a day. 8 weeks after sowing plants were harvested.

3.2.Physiological and Nitrogen use efficiency (NUE) parameters

Chlorophyll fluorescence (Fv/Fm) was measured using a portable multimode chlorophyll fluorometer (Model, OS5P Opti-sciences, Inc. Winn Avenue Hudson, USA). Relative chlorophyll concentration of leaves was estimated using chlorophyll meter (soil plant analysis development: SPAD-502 Konica Minolta, Tokyo, Japan). Five readings were obtained for each leaf. Chlorophyll fluorescence and SPAD measurement were carried out 7 weeks (49 days) after sowing (Z 32).

After harvesting (eight-weeks-old seedlings: Z 37) two samples from each pot (shoots and roots) were dried and ground to serve for ions content determination and NUE measurement. Plants ions content (Na^+, K^+) was determined according to Pauwels et *al.* (1992), a standard flame photometer procedure (Model PFP7 Flame photometer,Jenway, Bibby Scientific Ltd,UK) was used. To determine tissue N concentration, plant material was digested using Kjeldahl procedure and N concentration was measured according to the Cataldo et *al.* (1974)

method. The NUE components were measured using the following formulas (Nugyen et *al.*, 2014):

$$\text{N use efficiency (NUE) or agronomical NUE} = \frac{\text{Total dry weight (g plant}^{-1})}{\text{Total N applied}}$$

$$\text{N uptake efficiency (NupE) or absorption NUE} = \frac{\text{Total N absorbed (g plant}^{-1})}{\text{Total N applied}}$$

$$\text{N utilization efficiency (NutE) or physiological NUE} = \frac{\text{Total dry weight (g plant}^{-1})}{\text{Total N absorbed (g plant}^{-1})}$$

3.3.Growth parameters

After applying treatments; plant height, number of tillers and leaves were measured weekly. 8-weeks-old plants were harvested. Shoots and roots for each treatment were weighted then dried to a constant dry weight.

3.4. Statistical analysis

Statistical analysis was performed using R software (R- 64 3.6.1). The significance of genotypes, N application, salinity levels and their interaction were analyzed using a linear variance analysis model (ANOVA $p < 0.05$). Multiple linear regression analysis (stepwise) was performed to identify the best traits explaining biomass production as a dependent variable. GraphPad Prism 8 program was used to establish figures.

4. Results

4.1. Chlorophyll Fluorescence and relative chlorophyll concentration

In general order, salinity decreased SPAD value by 8%. By supplying N to plants a slight increase (8.2%) in SPAD value was observed. The highest SPAD value (40) was observed in plants grown under control condition (+N/-NaCl), and there was no significant difference between the rest of treatments (SPAD value=34).

Genotype, salinity and N application affected chlorophyll fluorescence (Table 1). Significant interaction between salinity and N treatment was found. Results showed that the lowest chlorophyll fluorescence (Fv/Fm) was obtained in the sensitive landrace “Barley Medenine” while no significant difference was observed between the rests of genotypes. Under high N

condition, there was no significant reduction in PSII maximum efficiency (Fv/Fm) subjected to saline treatments, but a significant decrease in Fv/Fm caused by salinity was detected under low N treatment (Figure 1).

Table 1: Analysis of variance for SPAD value and maximum quantum efficiency of PSII (Fv/Fm). *: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$.

Source of variation	Df	SPAD	Fv/Fm
Genotype (G)	**3**	30.9	0.00455**
Salinity (S)	**1**	517.2***	0.06068***
Nitrogen (N)	**1**	547.8***	0.15020***
(G)*(S)	**3**	10.1	0.00130
(G)*(N)	**3**	25.7	0.00013
(S)*(N)	**1**	460.3***	0.03976***
(G)*(S)*(N)	**3**	12.8	0.00109
Residuals	**32**	16.0	0.00090

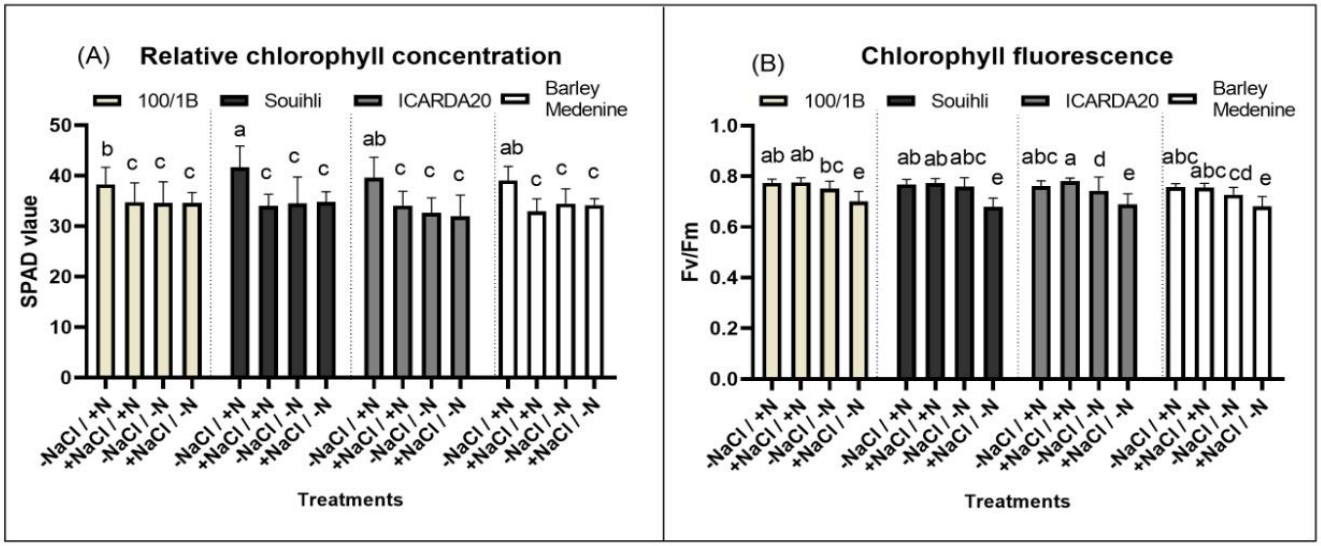

Figure 1: Effects of salinity and N treatments on relative chlorophyll concentration (A) and chlorophyll fluorescence (B) in four barley genotypes. Letters indicate significant differences between different treatments.

4.2. Nitrogen Use Efficiency components

To analyze how N delivery from root to shoot is affected under salt and nutrient treatment in the different genotypes, tissue N concentration in shoots and roots and NUE components were determined (Table 2). N supply significantly improved N concentration in shoots (53%) and roots (56%) (Figure 2 (A) and (B)). Under high N treatment, salinity decreased shoots N concentration by 10%, 15% and 24% in "Souihli", "ICARDA20" and "Barley Medenine"

respectively, whereas "100/1B" did not show variations. Non-stressed plants grown under low N condition showed a comparable shoot N concentration (1.75%); when NaCl added, shoot tissue N concentration decreased by 14%, 23% and 36% in "ICARDA20", "100/1B" and "Barley Medenine" respectively, while "Souihli" did not show variation. The shoots showed 3 times highest N concentration than the roots. Genotypes reacted differently vis-a-vis N concentration in roots; in fact when plenty of N is available, "100/1B" and "Barley Medenine" suffered of 25% of decrease in N roots concentration caused by salinity, whereas, "ICARDA20" proved its stability and "Souihli" showed a 20% of increase. When plants grown under limited N, salinity did not cause changes in tissue N concentration in roots.

Based on the Figure 2(C), very high significant increase of N uptake following N application, this increase reach 79% in "Souihli" and "ICARDA20" and 73% in "100/1B" and "Barley Medenine". All genotypes reacted similarly to salt treatment depending on N nutrition: in fact Salinity resulted in 23% and 29% of decrease of total N uptake in plants grown in high N and low N respectively.

Table 2: Analysis of variance for tissue N concentration in shoots ([N] shoots) and roots ([N] roots), total N uptake (N up), N uptake efficiency (Nup E), N utilization Efficiency (Nut E) and Nitrogen Use Efficiency (NUE). *: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$.

Source of variation	Df	[N] shoots	[N] roots	N up	Nup E	Nut E	NUE
Genotype (G)	**3**	1.23***	0.120***	177***	156.5***	436***	27.9***
Salinity (S)	**1**	1.89***	0.061.	352***	357.7***	1188***	13.5**.**
Nitrogen (N)	**1**	39.87***	5.668***	7020***	2809.5***	14495***	2390.8***
(G)*(S)	**3**	0.12***	0.071**	5	17.3	189***	2.4
(G)*(N)	**3**	0.52***	0.010	79***	18.1	14	18.9**
(S)*(N)	**1**	0.05.	0.004	105**	53.3*	332***	3.5
(G)*(S)*(N)	**3**	0.06**	0.071**	2	13.0	73***	1.3
Residuals	**32**	0.01	0.015	11	8.3	9	3.3

N uptake efficiency (NUpE) showed a very high significant difference between the two levels of N application; indeed, N supply led to reduce NUpE by 55% (Figure 2(D)). Plants exposed to salinity displayed lower NUpE than control plants; in fact salinity decreased NUpE by 24% independently of N treatments. A comparable efficiency in N uptake was observed in all genotype (22%), except "Barley Medenine" which revealed the lowest value (15%). Analysis of N Utilization Efficiency (NUtE) revealed that NUtE was reduced on average by 53.4% compared to low N condition. With plenty N supply, salt stress did not cause significant changes in all genotypes except "Barley Medenine" which showed 24% of increase. Whereas, different

responses of plants grown under N deficient condition was observed: without salinity all plants showed a comparable NUtE (57.5 mg DW/mg absorbed N), then NaCl addition increased NUtE by 36%, 23% and 14% in "Barley Medenine", "100/1B" and "ICARDA20" respectively, while no significant changes was noted in "Souihli".

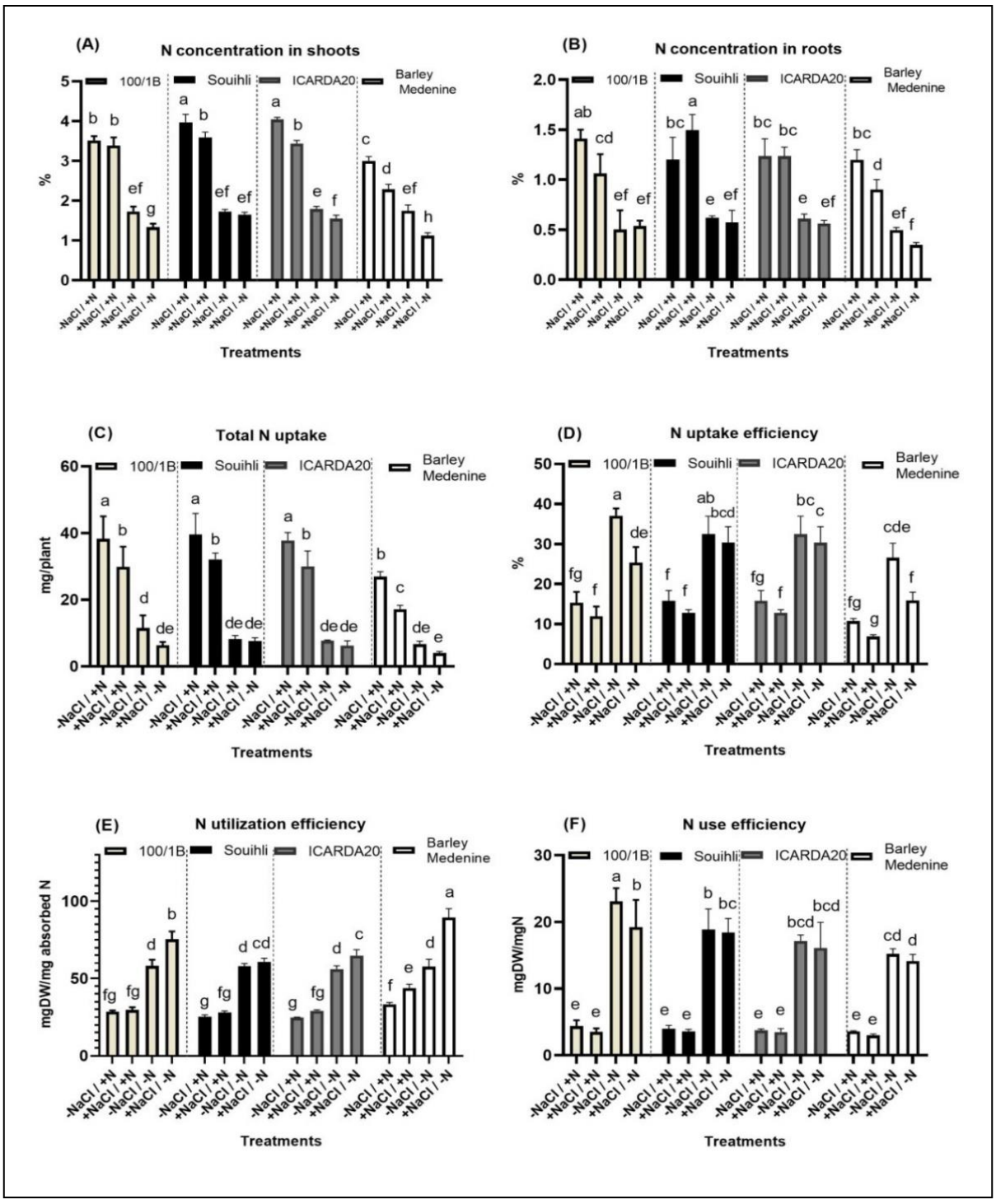

Figure 2: Effects of salinity and N treatments on tissue N concentration in shoots (A) and roots (B), total N uptake (C), N uptake efficiency (D), N utilization Efficiency (E) and Nitrogen Use Efficiency (F). Letters indicate significant differences between different treatments.

Results showed that NUE was highly influenced by N supply, it decreased by 80% with increasing N supply. Under high N treatment, no significant difference in NUE was obtained between genotypes. Whereas, under low N the highest NUE was obtained for the two tolerant genotypes "100/1B" (23 mg DW/mg N) and "Souihli" (19 mg DW/mg N).

4.3. Sodium (Na^+) and potassium (K^+) concentration and uptake

In order to study the impact of salt exposure on different barley genotypes fed with high and low N, the concentration of Na^+ ($[Na^+]$) and K^+ ($[K^+]$) in shoots and roots, Na^+ and K^+ uptake was analyzed. Results showed that $[Na^+]$ in roots and shoots increased with salinity by 60% (Figure 3 (A)). On the other hand, under high N treatment, potassium $[K^+]$ in roots decreased by 29% , 20%, 8% and 6% in "ICARDA20", "100/1B", "Souihli" and "Barley Medenine" respectively; whereas 17% of reduction was observed in plants shoots (Figure 3 (B)). N fertilizer had a consistent impact on $[Na^+]$ and $[K^+]$ especially in roots. In fact, N fertilizer mitigated the increase of [Na+] in roots from 73% under low N to 42% under high N. Similar effect was observed in shoots where N treatment attenuated the increase of $[Na^+]$ from 63% under low N to 57% under high N. N supply improved $[K^+]$ in both saline conditions: an increase of 36% in roots and 27% in shoots was observed under control conditions, whereas under salinity the improvement was around 31% and 29.5% in roots and shoots respectively. Interestingly the improvement of shoot $[K^+]$ by N application was 35% in "ICARDA20", while it was 27% in "Souihli" and "100/1B"; and 24% in "Barley Medenine".

Table 3: Analysis of variance for tissue Na^+ concentration in roots ($[Na^+]$ roots) and shoots ($[Na^+]$ shoots), K^+ concentration in roots ($[K^+]$roots) and shoots ($[K^+]$ shoots), Na^+ uptake and K^+ uptake.

*: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$.

Source of variation	Df	$[Na^+]$ roots	$[Na^+]$ shoots	$[K^+]$ roots	$[K^+]$ shoots	Na^+ uptake	k^+ uptake
Genotype (G)	**3**	8**	66***	270***	451***	7.6	822***
Salinity (S)	**1**	5612***	3924***	385***	1680***	1204.6***	2642***
Nitrogen (N)	**1**	59***	1	3300***	5461***	1036.3***	23823***
(G)*(S)	**3**	57***	36***	12***	2	16.1*	74
(G)*(N)	**3**	28***	2	125***	91***	2.4	171*
(S)*(N)	**1**	540***	19***	96***	21**	77.9***	557**
(G)*(S)*(N)	**3**	34***	1	85***	1**	1.7	5
Residuals	**32**	2	1	1	2	5.1	58

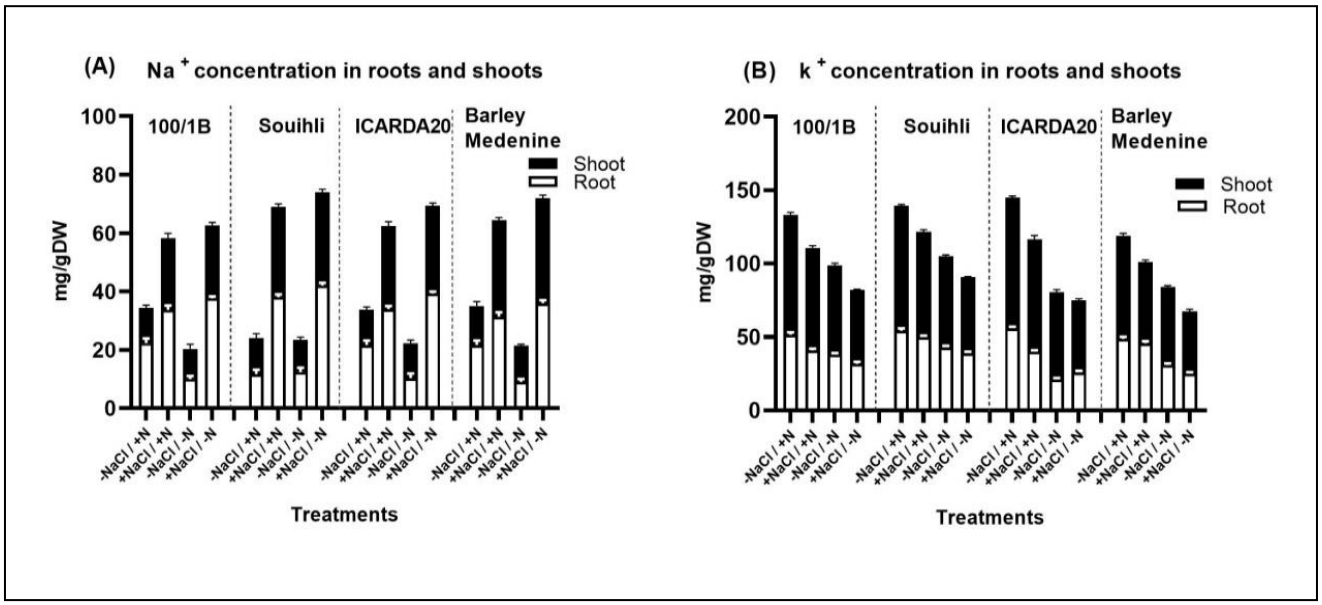

Figure 3: Effects of salinity and N treatments on tissue Na^+ concentration in shoots and roots (A) and K^+ concentration in shoots and roots (B).

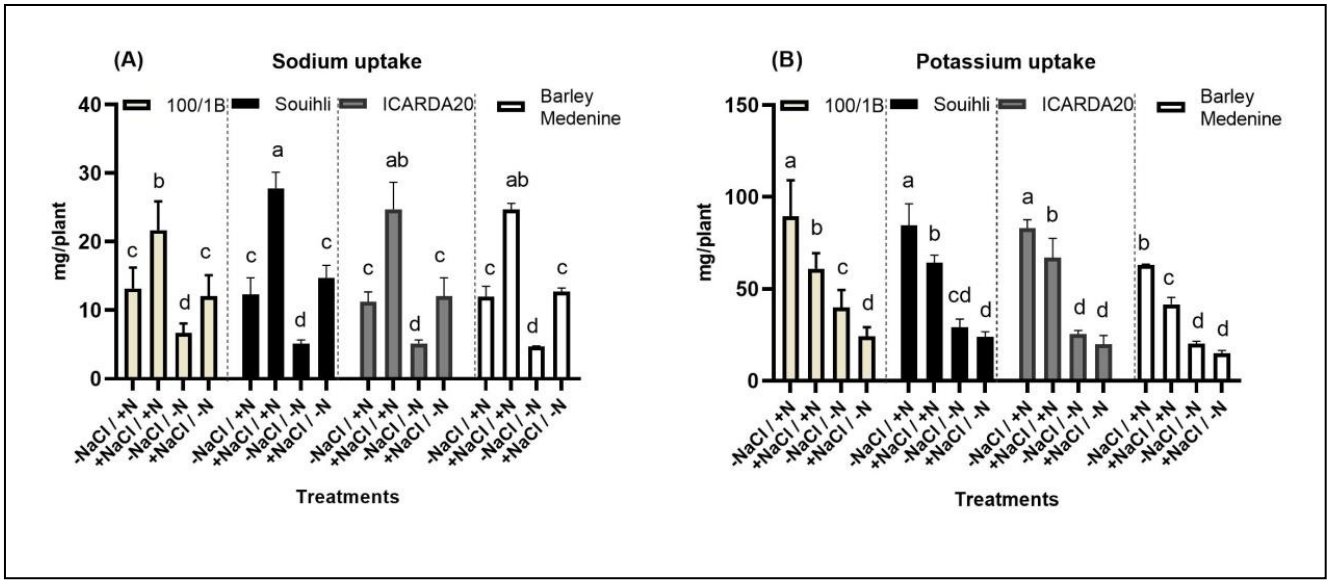

Figure 4: Effects of salinity and N treatments on tissue Na^+ uptake (A) and K^+ uptake (B).

The average of K^+ uptake was reduced by 27% under salinity (Figure 4(B)). N application through increasing [K^+] and dry weight increased K uptake by 65%. Under high N, salinity increased Na+ uptake by 50%; while a more important increase (58%) was observed under low N (Figure 4(B)).

4.4. Growth parameters

Because Nitrogen application, as it is expected, has a direct impact on growth, we analyzed vegetative growth in salt-treatment in dependence of N nutrition (table 4).

Table 4: Analysis of variance for fresh weight aerial part (FWAP), dry weight aerial part (DWAP), and fresh weight root part (FWRP), dry weight root part (DWRP).

*: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$

Source of variation	Df	FWAP	DWAP	FWRP	DWRP
Genotype (G)	3	2.5*	0.0714**	0.021	0.00091
Salinity (S)	1	59.3***	0.1098**	6.338***	0.08594***
Nitrogen (N)	1	385.2***	2.5374***	3.818***	0.03408*
(G)*(S)	3	0.3	0.0151	0.009	0.00215
(G)*(N)	3	0.2	0.0032	0.060	0.00985
(S)*(N)	1	59.7***	0.0146	3.494***	0.01446
(G)*(S)*(N)	3	1.4.	0.0013	0.065	0.00048
Residuals	32	0.6	0.0108	0.196	0.00588

The results showed that the tolerant "100/1B" produced more biomass than other genotypes. Salinity caused a fresh weight decrease in both aerial (32%) and roots part (27%). The negative effect of salinity was also significant for dry biomass that decreased by 13% in shoots and 26% in roots. N fertilizer improved fresh (65%) and dry shoot biomass (50%) (Figure 5); this improvement could be related to the important root development. In fact, N application increased root biomass by around 20%. N addition alleviated the decrease of shoot biomass caused by saline stress: NaCl treatment decreased shoot biomass by 40% under high N condition while this reduction was around 73% under low N condition.

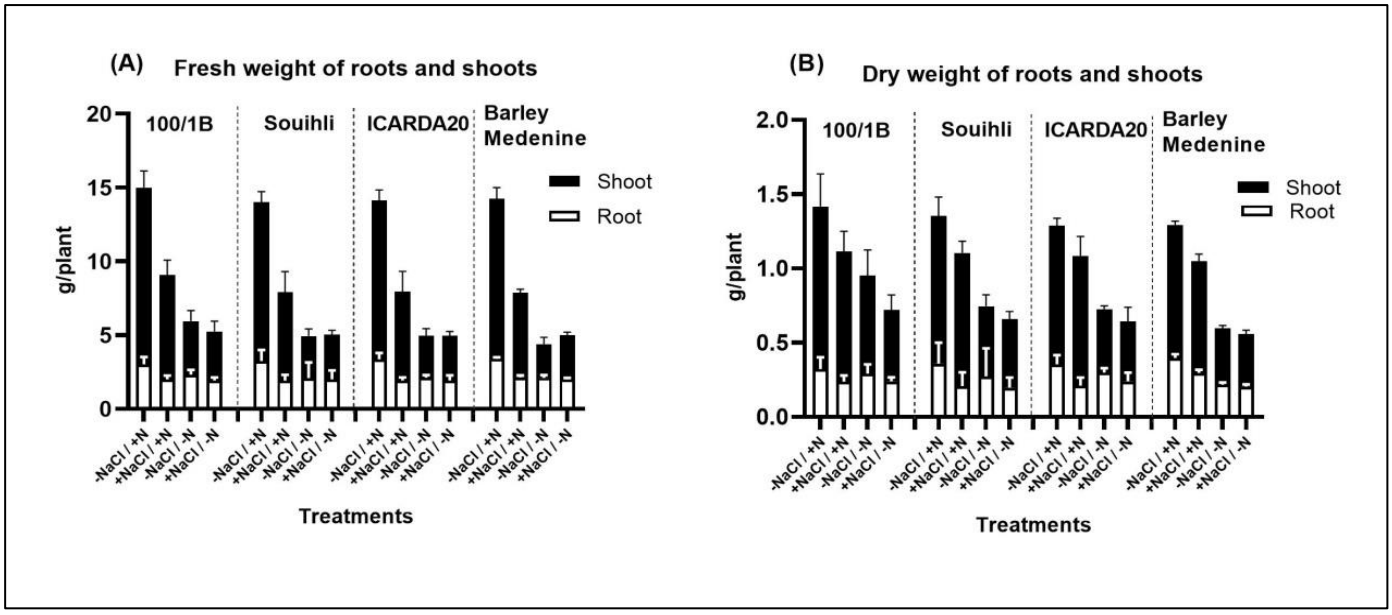

Figure 5: Effects of salinity and N treatments on fresh (A) and dry (B) weight of aerial and root part.five-weeks-old seedlings (35 days after sowing).

The effect of N deficiency was clear only in "Souihli" and "ICARDA20" which showed a height decrease compared to other genotypes. This decrease can reflect the sensitivity of "Souihli" and "ICARDA20" to N deficiency. A slight height enhancement by N fertilizer (7%) was observed 2 weeks after N supply (Table 5). Seven-weeks-old seedlings (49 days after sowing), plant height was significantly reduced by salinity (11%) depending on genotypes: reduction was around 5.2%, 10%, 12% and 17% in "ICARDA20", "Souihli", "100/1B" and "Barley Medenine" respectively.

Table 5: Genotype and treatment effects on plant height 5, 6 and 7 –weeks- old seedlings (WOS), number of tillers (Tiller nb) 6 and 7 –weeks- old seedlings and leaf area of barley genotypes. ANOVA analysis is presented in the lower panel.

*: significant at $p < 0.05$; **: significant at $p < 0.01$; *** significant at $p < 0.001$.

		Height (5 WOS)	Height (6 WOS)	Height (7WOS)	Tiller nb (6 WOS)	Tiller nb (7 WOS)	Leaf area (7 WOS)
Genotype (G)							
"100/1B"		21,84 a	31,92 a	37,55 a	0,6 b	1,53 c	28,16 a
"Souihli"		20,37 a	30,07 b	35,12 b	0,85 a	1,86 ab	23,91c
"ICARDA20"		20,35 a	29,89 b	35,57 b	0,9 a	2,03 a	25,83 b
"Barley Medenine"		21,44 a	30,19 b	34,3 b	0,63 b	1,58 bc	23,16 c
Salinity (S)							
low Salinity		21,46 a	30,94 a	37,87 a	0,8 a	1,89 a	29,20 a
high Salinity		20,59 a	30,15 a	33,68 b	0,68 b	1,71 b	21,33 b
Nitrogen (N)							
low N		20,8 a	29,45 b	32,73 b	0,35 b	0,99 b	22,41 b
high N		21,19 a	31,59 a	38,54 a	1,15 a	2,51 a	28,125 a
ANOVA							
	df						
Genotype (G)	3	34.33	53.49*	114.3***	1.19**	3.37**	59.9***
Salinity (S)	1	45.73	37.24	1047.2***	0.93*	0.34*	744.2***
Nitrogen (N)	1	9.01	275.20***	2024.2***	37.60***	139.54***	391.0***
(G)*(S)	3	27.72	35.54.	52.7*	1.12**	1.16	33.4***
(G)*(N)	3	112.93**	15.30	28.9	1.07*	1.65.	8.5**
(S)*(N)	1	44.92	74.18*	262.6***	10.19***	10.97***	2.5
(G)*(S)*(N)	3	14.90	3.46	12.8	0.38	0.96	3.3
Residuals	224	22.20	14.68	15.4	0.29	0.70	1.6

On the other hand seedlings height was significantly increased by N fertilizer (15.5%) depending on salinity: increase was 20% under non saline condition and 11% under salinity. Plant height in "100/1B" landrace is generally higher than other genotypes. When plants grown under high N treatment the reduction in number of tillers caused by salinity was 18%, while it was 30.5% under low N treatment. N addition improved the number of tillers by 70% in "Souihli" and 63% in the rest of genotypes (Table 5). Leaf area was significantly decreased under salinity (27%); this decrease was variable between genotypes (Table 5). In fact, the tolerant "100/1B" and the improved "ICARDA20" were the less affected by salinity and reduced leaf area by 13% and 23% respectively, while in "Barley Medenine" and "Souihli" reduction was around 33% and 39% respectively. N supply increased the average of leaf area by 20.3%. Based on the significant interaction between Nitrogen and genotypes, results showed that the improvement of leaf area by N fertilizer was relative to genotypes, in fact, 28%, 24%, 17% and 13% were the leaf area improvement by N fertilizer for the genotypes "Barley Medenine", "Souihli", "ICARDA20" and "100/1B" respectively.

4.5. Stepwise analyses of biomass production

A multiple linear regression (stepwise method) was performed to analyze biomass production variations as a dependent variable. Regression analysis revealed that N concentration in root and the uptake of Na^+ and K^+ were selected to be the most effective traits explaining biomass production. The analysis showed that 83% of biomass production variation was explained by this model (Table 6).

Table 6: Multiple linear regressions analyses (stepwise) explaining the variation biomass production in barley genotypes. Levels of significance: ***P < 0.001; **<0.005.

Dependent variable	Variable chosen	Estimate	Standar Error	t	Significance	R2	Adjusted R2
	constant	0.25750	0.51646	0.499	0.6		
Biomass	root [N]	2.83432	1.05876	2.677	0.010*	0,84	0,83
production	K+ uptake	0.08985	0.01449	6.201	0.00***		
	Na+ uptake	-0.08103	0.03179	-2.549	0.014*		

Final model: Biomass production= 2.8 root N concentration +0.089 K^+ uptake - 0,08 Na^+ uptake + 0.257

The model revealed that major biomass production variation was explained by root N concentration variability with a slope of 2.8; it was also positively proportional to K^+ uptake and negatively proportional to Na^+ uptake. In other terms, as root N concentration and k+ uptake increased, biomass

production was found to be increased; while as Na+ uptake increased, biomass production tends to be decreased.

5. Discussion

Understanding the relationship between N fertilization and salinity is of great agronomic and economic interest for crop production (Chen et *al*., 2010). Our study proved that photosynthetic rate, relative chlorophyll concentration, N use efficiency components, Na^+ and K^+ concentration and uptake, plant height, and the number of tillers were significantly influenced by the interaction of salinity and N fertilization. Under salinity Na^+ and Cl^- cause interference with uptake, translocation, and assimilation of essential plant nutrients such as nitrate (NO_3^-), ammonium (NH_4^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), and sulfate (SO_4^{2-}), leading to disturbance of ion homeostasis and growth process.

In order to prevent the adverse effect caused by salinity, plants involved a variety of salt tolerance mechanisms to maintain the metabolic functions. Our findings showed the necessity of N fertilizer under salinity to maintain the physiological processes which are in direct relation with plant growth and productivity. Under salinity, N supply enhanced leaf area and stabilized the photosynthetic rate, which in turn resulted in attenuated reduction caused by salinity in growth parameters (biomass production, height, and tillers number). In this saline condition, N supply mitigated the increase of Na^+ tissue concentration and Na^+ uptake. In addition, results showed that N uptake in barley plants were reduced under salinity probably due to the antagonism effect between NO_3^- and Cl^- (Parida and Das 2004, Chen et *al*., 2010). This reduction was significantly attenuated when N was applied. In fact, nitrate application can reduce Cl^- uptake and accumulation; and alleviate the negative effect of salt stress on plants (Flores et *al*., 2012). The enhancement caused by N fertilization was mostly due to the amino acids which is an essential component of proteins that construct cell and plant tissues. They play a key role in plant development, yield, and grain protein accumulation (Perchilik and Tegeder, 2017); furthermore, they counterbalance the raised osmotic potential from saline solution and protect cells by scavenging reactive oxygen species (ROS) (Flores et *al*., 2012). N uptake efficiency, N utilization efficiency and N use efficiency were decreased with N addition; similar results were reported by Perchilik and Tegeder (2017). An important genotypic variability was observed: the tolerant landrace "100/1B" showed the highest NUE, K^+ uptake, and the lowest Na^+ concentration. On the other hand, the lowest NUE and K^+ uptake, and the highest Na^+ concentration were observed in the sensitive "Barley Medenine". Our findings support the hypothesis that NUE, Na^+ concentration, and K^+ uptake can be a useful index of

barley tolerance against salinity. The competitive relation between sodium and other nutrient ions necessary for plants can explain the lowest K^+ uptake observed in "Barley Medenine". K^+ decrease is generally accompanied by Ca^{2+}, Mg^{2+}, and Mn^{2+} decrease, and results in cells ionic imbalance which affects whole plant physiology. Na^+ can inhibits the absorption of K^+ in barley plants directly through competition for the same type of transporters, or indirectly by osmotic effect and the inhibition of root growth. In all genotypes and especially "Barley Medenine" which accumulate Na^+ in the place of K^+, protein synthesis and the activity of several enzymes, especially in photosynthetic tissues was mainly disturbed; because a high intracellular concentration of K^+ plays the role of an important reaction cofactor (Bounaqba, 1998). Thus, the replacement of K^+ by Na^+ at the level of the active sites of proteins is very harmful to most enzymes. It induces a change in the spatial conformation of the enzyme and the loss of its protein function, leading to cellular dysfunctions. This disorder caused by the toxic ions Na^+ and Cl^- reduced plant growth and also decreased relative chlorophyll concentration and chlorophyll fluorescence which was markedly observed in "Barley Medenine" genotypes. Some previous studies reported that growth reduction under salinity is a consequence of the decrease in photosynthetic capacity in plants (Netondo et *al.*, 2004, Song et *al.*, 2019). In turn, photosynthetic capacity decrease can be attributed to the decrease of photosynthesis area which was decreased by 27%. Our study showed that N deficiency limited plant growth more than salinity.

6. Conclusion

This work proved that N supply increase plant tolerance through the triggering of tolerance mechanisms and the improvement of nutritional status of barley plants. N fertilization can be a great strategy to enhance plant performance in saline environment. This study revealed that N deficiency threatens plant growth more than salinity. In our study, barley plants were only subjected to 150 mM, thus the barley responses to higher salinities and different N levels may need further study. In addition, further studies in enzymes involved in N metabolism and antioxidant defense system are required to better understand the plant regulation system under abiotic stress.

Chapter IV: Salt Stress Induces Differentiated Nitrogen Uptake and Antioxidant Responses in Two Contrasting Barley Landraces from MENA Region

Published in Agronomy-Basel Journal

1. Abstract

The interaction between salinity and nitrogen metabolism has been investigated in two barley landraces, one tolerant ("100/1B") and one susceptible to salinity ("Barley medenine") from the Middle East and North Africa (MENA) region. Barley plants were exposed to 50 mM NaCl for 7 days; then, salinity was increased to 150 mM NaCl in the presence (10 mM) or limitation (1 mM) of ammonium as a nitrogen source. Upon salinity, "100/1B" was shown to support N assimilation by enhancing the glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT) cycle under high N, and the stimulation of the glutamate dehydrogenase (GDH) pathway under low N treatment. In "Barley medenine", salinity reduced the GS/GOGAT cycle, and increased GDH activity. Upon salinity, Heat Shock Proteins 70 and phosphoenolpyruvate carboxylase (PEPC) remained unchanged in "100/1B", while they decreased in "Barley medenine". The tolerance degree is a determining factor in enzymes' occurrence and regulation: exposed to salinity, "100/1B" rapidly increased ascorbate peroxidase (APX) and PEPC activities, while this was delayed in "Barley medenine". Salinity increased cytosolic glucose 6-phosphate dehydrogenase (cyt-G6PDH) levels in "100/1B", while "Barley medenine" showed a decrease in G6PDH isoforms. Correlation analyses confirm GOGAT was related to G6PDH; GDH and APX with PEPC in "100/1B" under moderate salinity; severe salinity correlated GDH with G6PDH and PEPC. In "Barley medenine" under salinity, GOGAT was correlated with G6PDH, while APX showed a relation with PEPC. Therefore, specific enzymatic activities and occurrence can be used to determine stress responsiveness of different landraces. We suggest that the rapid increase in G6PDH, APX, and nitrogen assimilation enzymes activities represents an index of tolerance in "100/1B" and a stress symptom in "Barley medenine".

Keywords: salt stress; nitrogen metabolism; oxidative stress response; G6PDH; GDH; GS/GOGAT

2. Introduction

High salinity is one of the most widespread abiotic stresses affecting plant physiology, growth, and development (Martinez-Atienza et *al.*, 2007 ; Ruggiero et *al*., 2019). Remarkable reduction in cereals productivity was recurrently reported inrecent decades by abiotic constrains, namely salinity and drought (Lobell et *al*., 2011; Halford et *al*., 2015). Exposure to an excess of salts triggers different metabolic changes by modifying the balance between nutrient availability and plant requirements, and inducing associated constraints, namely osmotic, ionic, and oxidative stresses (Bartels et *al*., 2005). These conditions disturb cells water potential, disrupt nutrient availability caused by competitive uptake, and generate reactive oxygen species (ROS) (Ashraf et *al.*, 2018). One of the most important mechanisms conferring acclimation and tolerance of plants to abiotic stresses and salinity is the regulation of ROS levels. This stress response is regulated by a specific scavenging complex, composed by both enzymes and non-enzymatic antioxidant compounds (Jallouli et *al*., 2019). Critical enzymes involved in ROS detoxification are ascorbate peroxidase (APX, E.C. 1.11.1.11), catalase (CAT, E.C. 1.11.1.6), superoxide dismutase (SOD, E.C. 1.15.1.1), and glutathione reductase (GR, E.C. 1.8.1.7) (Gill et *al.*, 2010; Caverzan et *al*., 2012). Furthermore, an assisting role as reductants' supplier has been recently proposed for glucose 6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49), which provides additional NAPDH for scavenging enzymes upon abiotic stresses such as salinity and drought (Landi et *al*., 2017; Zhao et *al*., 2015). On the other hand, salinity affects nitrogen (N) metabolism inducing nutritional imbalances (Ashraf et *al.*, 2018). N is a crucial plant nutrient required for growth and development (rghavani et *al.*, 2017). Therefore, the interaction between salinity and N metabolism is particularly complex, because of the antagonism in plant response under these combined interactions. N supply improves nutritional plant status and alleviates toxicities of abiotic stress (Singh et *al.*, 2016). In this context, crucial roles were played by enzymes such as phosphoenolpyruvate carboxylase (PEPC, E.C. 4.1.1.32), which regulate the connection between carbon and N metabolism for the replacement of carbon skeletons and the replenishment of the tricarboxylic acid cycle; G6PDH plays a pivotal role in reductants provision also for enzymes involved in N metabolism and assimilation (Esposito, 2016). Furthermore, N availability showed contrasting roles in abiotic stress susceptibility/tolerance: deficiency of this nutrient could reduce water loss but N transporters, namely A*t*NRT1.1 (A*t*1g12110), A*t*NRT1.8 (A*t*4g21680), and CLCA (A*t*5g40890), showed positive effects on plant response to abiotic stresses (De Angeli et *al*., 2016; Landi et *al*., 2017).

Nowadays, the enhancement of crop yields upon high saline environments led to a renewed

interest for plant physiology researchers. Mediterranean regions, especially arid and semi-arid areas from Southern Europe and Northern Africa, were usually characterized by ecosystems subjected to multiple stress conditions characterized by contemporary onset of excess of salinity and/or water scarcity, heat, and nutrients (Landi et *al*., 2019). Therefore, farmers throughout centuries have developed a number of traditional varieties. These genotypes, generally described as landraces, showed the ability to tolerate environmental changes maintaining unaltered yields (Ruggiero et *al*., 2019; Dwivedi et *al*., 2016). In the Middle East and North Africa (MENA) region, barley represents a critical agronomic resource in semi-desert environments, especially in developing countries where this crop is a critical component of cereal rotation, providing a stable source to sustain smallholder farmers, and often replaces wheat or other cereals in more arid areas (Hammami et *al*., 2017). On the other hand, barley is the fourth most produced cereal in the world (FAO, 2017), representing one of the main sources of carbohydrates in developing countries, due to its natural stress tolerance, thus supporting small farmers in many arid areas (Hammami et *al*., 2017, Shen et *al*., 2016; Lee et *al*., 2020). Furthermore, barley is an excellent model organism to investigate abiotic and biotic stress resistance, being tolerant to different environmental stress (Gürel et *al*., 2016). It is worth pointing out that the identification of novel alleles QTL and peculiarities from unexploited genotypes is a central challenge for researchers, in order to improve crop productivity in vulnerable environments and provide new tools to increase crop yields (Dwivedi et al., 2016; Tuberosa, 2010). For example, genes such as Hs*C*BL8 (calcium-sensor calcineurin B-like) and Hs*C*IPKs—identified in Tibetan genotypes of *Hordeum spontaneum*—were overexpressed in rice, contributing to an increased tolerance to salinity, drought, and heavy metals (Guo et *al*., 2016). The aim of this paper is to investigate the effects of the interaction between salinity and N metabolism in two contrasting barley landraces. Our strategy is finalized to understand how the selected landraces respond to the detrimental effects induced by salt stress and how salinity regulates the N metabolism. To do this, biochemical activities of enzymes involved in responses to salinity and nitrogen assimilation have been studied. Our hypothesis is that landraces showing contrasting responses to salinity and nitrogen assimilation might reflect distinct regulations in their main metabolic pathways in order to adapt to environmental conditions. Therefore, specific enzymatic activities and protein occurrence can be identified as stress responsive sensors in different cereal genotypes; this possibility will be discussed under the light of the results obtained.

3. Materials and Methods

3.1. Plant Material and Growth Conditions

Two barley landraces, "100/1B" and "Barley medenine", which differ in their response to salinity,were chosen for this study. "100/1B" is a salt-tolerant landrace from Oman; the seeds were supplied by the Laboratory of Genetics and Cereal breeding—INAT, University of Tunis. "Barley medenine" is a salt-susceptible landrace selected by International Center for Agricultural Research in the Dry Areas (ICARDA) for "PRF sud project" in 2009; the seeds were obtained from the National Research Institute for Rural Engineering, Water and Forestry, Ariana, Tunisia in collaboration with the International Center for Biosaline Agriculture, Dubai, United Arab Emirates. Soil salinity in the origin area of "100/1B" and "Barley medenine" is 2 and 1.3 (dS m^{-1}), respectively (Hammami et al., 2017). Seeds were germinated for 7 days in the dark on moistened paper, then seedlings were grown in hydroponic solution in darkened plastic bottles at 20 °C, at 60–80% relative humidity, under a 16h-light/8h-dark regime, with approximately 180 µmol photons m^{-2} s^{-1}. The composition of the medium (modified Hoagland solution), continuously bubbled with air, has been previously described (Landi et *al.*, 2019). Hydroponic solutions were controlled for pH (6–6.5) and adjusted daily.

3.2. Nitrogen and Salt Treatments

After 7 days of hydroponic culture and before starting any treatments, 10 seedlings from each landrace were collected for analysis (-N/-NaCl). Then, plants were separated into 4 groups: Two control groups were grown without NaCl, upon low or high N supply with 1 and 10 mM of $(NH_4)_2SO_4$, respectively. The other two groups were grown under the same concentration of (NH4)2SO4 and subjected to moderate salt stress (50 mM NaCl), which was increased to severe stress (150 mM NaCl) after 7 days. Seedlings were collected 3 and 7 days after moderate NaCl stress (50 mM NaCl); further samples were collected after 1 and 3 days under imposing severe stress (150 mM NaCl). This experimental scheme was designed to simulate field conditions where salinity sequentially increases from low to high levels. These degrees of salinity (50 and 150 mM NaCl) allow investigating of both tolerance and adaptation to this abiotic stress. Sampling days have been selected based on previous biochemical investigation on similar enzymes upon abiotic stress (Landi et *al*., 2017; Landi et *al*., 2019), which indicated 3 and 7 as the most stressful days. The whole experimental design is depicted in Figure 1.

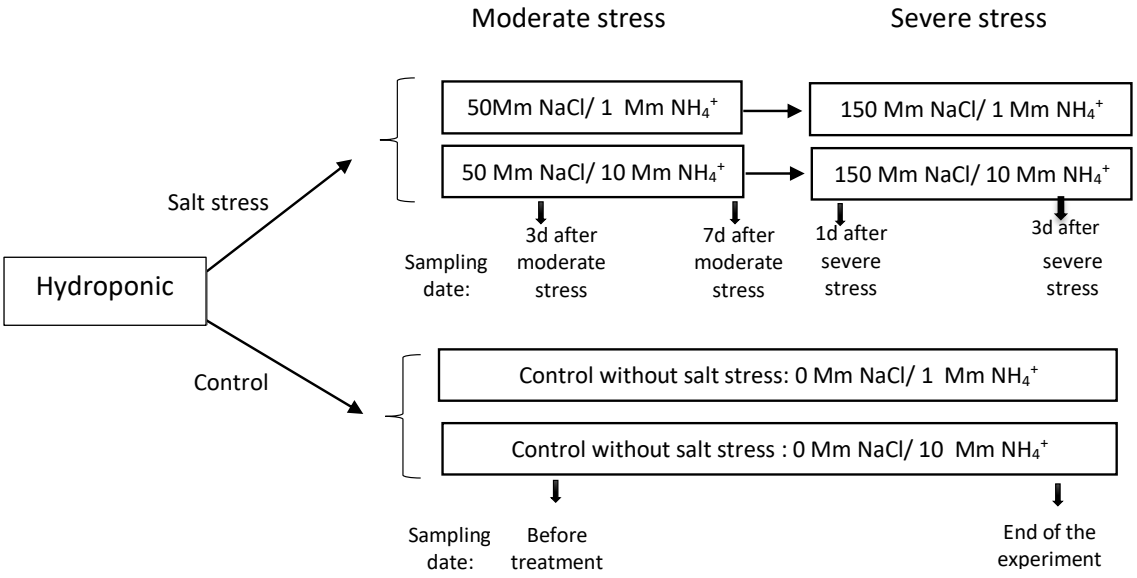

Figure 1: Scheme of the adopted experimental strategy. Stressed plants treated by low and high N were collected after 3 and 7 days of moderate stress (50 mM NaCl); then, a severe stress (150 mM NaCl) was applied and plants were collected after further 1 and 3 days. Control plants (without NaCl) were collected before any treatment, and after N application at the end of experiment

3.3. Enzyme Extraction and Activities Determination of NADH-GOGAT and NADH-GDH

NADH-GOGAT and NADH-GDH were extracted by grounding 300 mg of barley leaves in the same buffer containing 1 mL of 100 mM KH2PO4 buffer (pH 7.5), 2 mM EDTA, 2 mM dithiothreitol (DTT), and plant-specific proteases inhibitor cocktail (Sigma-Aldrich, St. Louis, USA). GOGAT and GDH assays were measured by following the oxidation of NADH at 340 nm for 10 min as described by Groat and Vance (1981) and Singh and Srivastava (1986) respectively. Specific activity was expressed as nmol NADH mg^{-1} protein min^{-1}. The reaction mixture for NADH-GOGAT assay contained 100 mM KH_2PO_4 buffer (pH 7.5), 18.75 mM2-oxoglutarate, 15 mM aminooxyacetate, 0.15 mM NADH, 7.5 mM L-glutamine, and extract. GDH activity was measured using 100 mM KH_2PO_4 buffer (pH 7.5), 200 mM $(NH_4)2SO_4$, 0.15mMNADH, 2.5mM2-oxoglutarate, and extract. Enzymatic activities were determined spectrophotometrically using a Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, CA,USA) at 25 °C. For all enzymatic assays, the protein concentration in the samples was measured using the Bradford method.

3.4. Enzyme Extraction and Activities Determination of G6PDH, APX, and PEPC

G6PDH, APX, and PEPC activities were extracted by grounding 300 mg of barley leaves in the same extraction buffer containing 50 mM Tris-HCl, pH 8.0, 5 mM MgCl2, 4 mM EDTA, 10%

glycerol, and plant-specific proteases inhibitor cocktail (Sigma-Aldrich, St. Louis, USA). (1) G6PDH was assayed as previously described by Castiglia et *al.* (2015). NADP+ was determined at 340 nm for 2–10 min. The reaction mixture contained 50 mM Tris-HCl buffer (pH 8.0), 5 mM $MgCl_2$, 0.15 mM $NADP^+$, 3 mM G6P, and 100 µL of extract. Enzyme activity was expressed as nmol-reduced NADP+ min^{-1} mg^{-1}protein. (2) APX activity was determined following the oxidation of ascorbic acid at 290 nm for 5–10 min. APX assay was carried out as described by Nakano and Asada (1981); the assay mixture contained 30Mm potassium phosphate buffer (pH 7.5), 1 mM EDTA, pure hydrogen peroxide H_2O_2, 5 mM ascorbic acid, and extract. The enzyme activity was expressed as µmol of ascorbic acid min^{-1} mg^{-1} protein. (3) PEPC was assayed by following the method of Fontaine et *al*. (1999) with some modifications. For PEPC determination, a reaction coupled to malate dehydrogenase (MDH) is required. PEPC activity was measured by following NADH oxidation at 340 nm for 10 min. The reaction was assayed at 25 °C, in a mixture containing 100 mM Tris–HCl (pH 8.2), 20 mM $MgCl_2$, 100 mM $NaHCO_3$, 0.2 mM NADH,MDH (3µL/mL Tris HCl pH 8.2); 10 mM PEP was used to start the reaction; a control without PEP was prepared for each assay. Enzymatic activities were determined spectrophotometrically using a Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, USA) at 25 °C. For all enzymatic assays, the protein concentration in the samples was measured using the Bradford method.

3.5. Western Blotting

For Western blotting analysis, proteins were extracted as described previously (Section 3.4) and then, separated using electrophoresis SDS-PAGE. Polypeptides were transferred on a Hybondmembrane (Ge Healthcare, Chicago, IL, USA) using a Transblot turbo transfer system (Biorad, Hercules, CA, USA). The membrane was incubated with primary G6PDH antibody raised against potato cytosolic (cyt), chloroplastic (P1), and plastidial (P2) G6PDH isoforms (Wendt, 2000); barley Fd-GOGAT (Pajuelo et *al*., 2000); HSP70 (cytosolic and chloroplast—Agrisera, Vännäs, Sweden) and PEPC from antiserum from *Amarantus edulis*. After incubation of the membrane with secondary antibodies, cross-reacting polypeptides were identified by enhanced chemiluminescence using the Western Brigh tQuantum kit (Advansta-Aurogene, Roma, Italy). Imageswere acquired by the BioRad Chemidoc system/Quantity One software (BioRad, Hercules, CA, USA).

3.6. Statistical Analyses

R software (R- 64 3.6.1) was used for statistical analysis. The significance of saline stress, N treatment, and duration of salt stress single effects and their interaction were analyzed using a

linear variance analysis model (ANOVA $p < 0.05$). Then, means were compared using Duncan's test. Correlation analysis using Pearson's parametric correlation test was performed to determine the correlation coeffcients for all possible pairs of columns in the data table.

4. Results

4.1. Contrasting barley landraces showed diversified stress and antioxidant responses

The ability of two contrasting barley landraces to respond to a combination of salinity and nitrogen starvation stresses were analyzed by the biochemical investigation on N metabolism and antioxidant enzymes. The different behavior in salt stress condition and N concentrations was clearly depicted in Figure 2, which showed leaves from these two landraces under experimental conditions. "100/1B" leaves showed a reduced damage induced by salinity, while "Barley Medenine" landrace showed strongly stressed leaves, both upon high and low N.

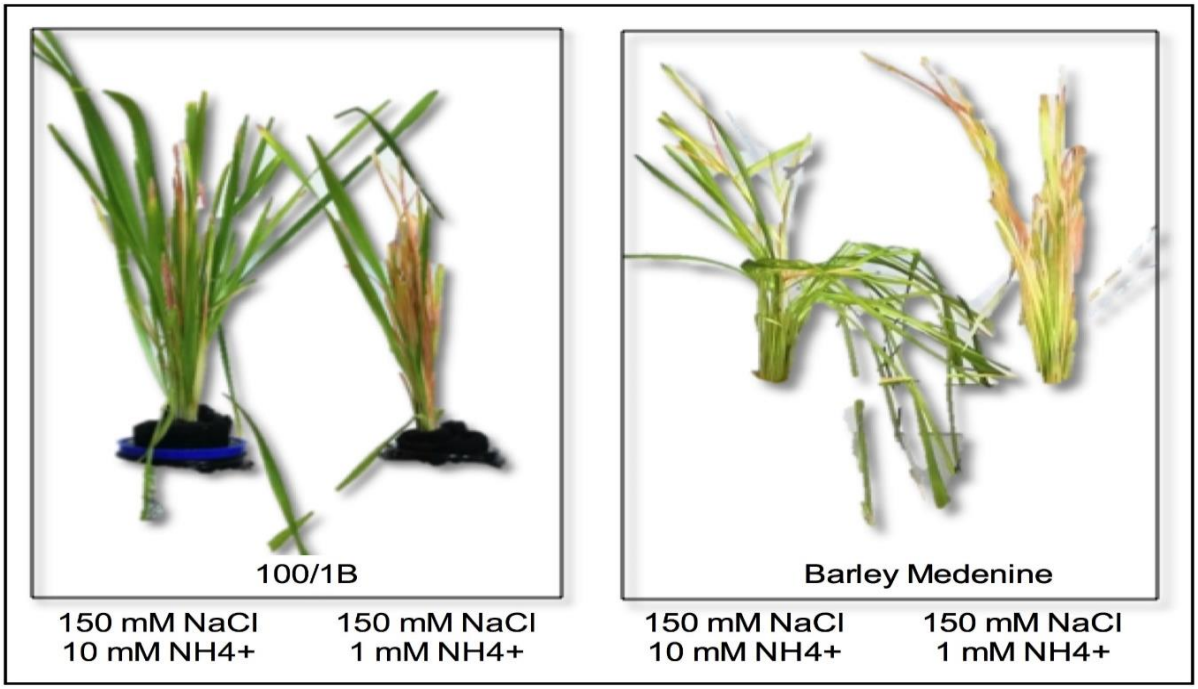

Figure 2: Effects of salinity and N supply on "100/1B" and "Barley Medenine" leaves.

The salt tolerant landrace "100/1B" showed a reduced involvement of APX upon salinity under lowN conditions (Figure 3). A rapid APX activity increase of about 50%, related to severe salt stress, was observed in "100/1B" plants grown in high N, whereas under low conditions, plants showed an APX activity essentially unchangedby salinity. The presence of an adequate source of N (10 mM NH_4) induced an increase of APX activity upon 150 mM of NaCl.

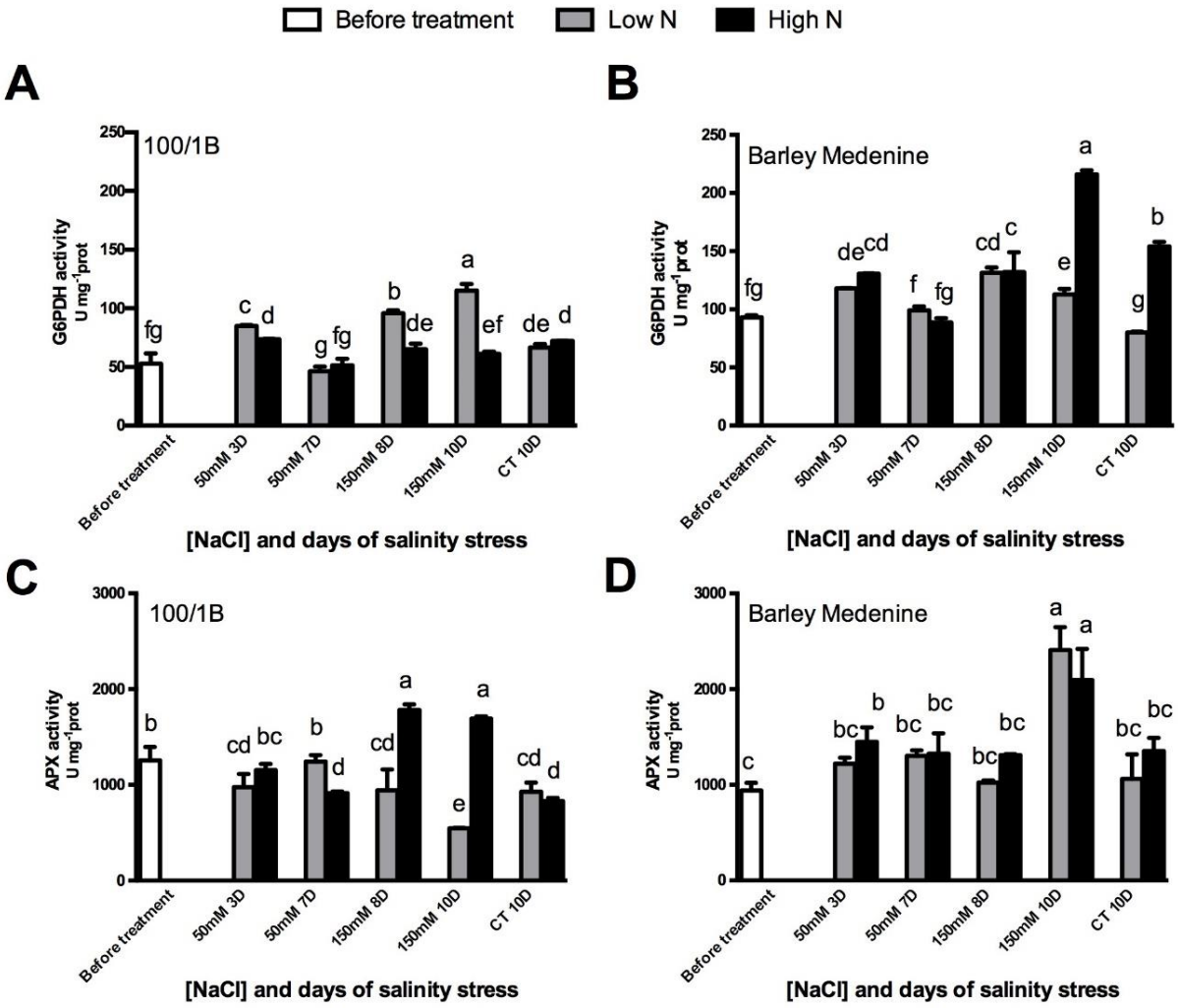

Figure 3: Effects of salinity and N concentration on G6PDH and APX enzymatic activities in barley plants growth in hydroponic system. Levels low N concentrations are in grey bars highconcentrations are in black bars. Legend: (BT), Before treatment; (50 mM 3D), 3 days of moderate stress; (50 mM 7D), 7 days of moderate stress; (150 mM 8D), 7 days in 50 mMNaCl and 1 day in 150 mMNaCl (severe stress); (150 mM 10d) 7 days in 50 mMNaCl and 3 days in 150 mMNaCl (severe stress); (CT 10D) control treatment for 10 days.Letters indicate significant differencesbetween different treatments. ($P < 0.05$, ANOVA: Duncan test).

The involvement of G6PDH in "100/1B" appeared to be mainly related to N assimilation by a significant increase upon N low concentration condition compared with N high concentration. On the other hand, susceptible "Barley Medenine" showed increased activities of APX and G6PDH upon salinity both in N high and low N concentration. It should be underlined that the sensitive landrace "Barley Medenine" showed higher G6PDH activities than "100/1B" when expressed on a mg^{-1} prot. The maximum activity in low N plants was observed after 24h of 150

mMNaCl exposure, while high N induced a marked increase up to 216 nmol min^{-1} mg^{-1}prot after 3 days of severe stress.

In order to investigate the contribution of different G6PDH isoforms antibodies constructed against cytosolic (Cyt-G6PDH), chloroplastic (P1-G6PDH) and plastidial (P2-G6PDH) were used by western blotting (WB – Figure 4). Results showed an increase in cyt-G6PDH abundance upon 150 mM NaCl in high N "100/1B" plants while an evident decrease was observed in low N plants (Figure 4). P1-G6PDH remained unaffected at low N regimes in "100/1B" , and decreased under high N under both moderate and high salinity. Plastidic P2-G6PDH decreased in "100/1B" under low N and salinity and remained unaffected by salt stress under high N. Similar behaviors were observed in "Barley Medenine" . Finally, the degree of the stress conditions was monitored by the HSP70s occurrence (Figure 4). Upon moderate stress, cyt-HSP70 protein occurrence remained unchanged in low N conditions in "100/1B" while high salinity induced a sensible increase of cyt-HSP70 upon high N.By contrast, "Barley Medenine" showed a high stability in cyt-HSP70 abundance upon high N independently of NaCl concentration.Chl-HSP70 showed no differences in "100/1B" in each analyzed condition with the exception of an increase upon high salinity and high N after 10D. In "Barley Medenine" Chl-Hsp70 showed a decrease upon high salinity, independently by N supply.

4.2. N metabolism modifications upon salinity: The role of GOGAT and GDH

Salinity did not induce substantial changes in NADH-GOGAT activity both under high or low N in tolerant "100/1B" (Figure 5). The only exception was represented by the higher stress point (10 day, 150mM NaCl, 10 mM NH4) where the NADH-GOGAT activity showed a 22% increase.

Furthermore, GDH activity showed an 24% increase upon severe salt stress in low N after8 days. On the other hand, in "Barley Medenine" NADH-GOGAT showed reduced differences between controls and salt stressed conditions both upon low and high N concentrations. Contrarily, an evident increase of GDH activity was observed in the susceptible "Barley Medenine" in both low N and high N conditions; this increase was more evident underlow N. Fd-GOGAT occurrence was investigated using specific antibodies. As shown in figure 4, "100/1B" reported adecrease of Fd-GOGAT occurrence in low N grown plants under salinity; while under high N conditionsno differences were observed upon salt stress. Similar behavior in "Barley Medenine" , where only a more severe decrease in Fd-GOGAT occurrence was observed under 150 mMNaCl.

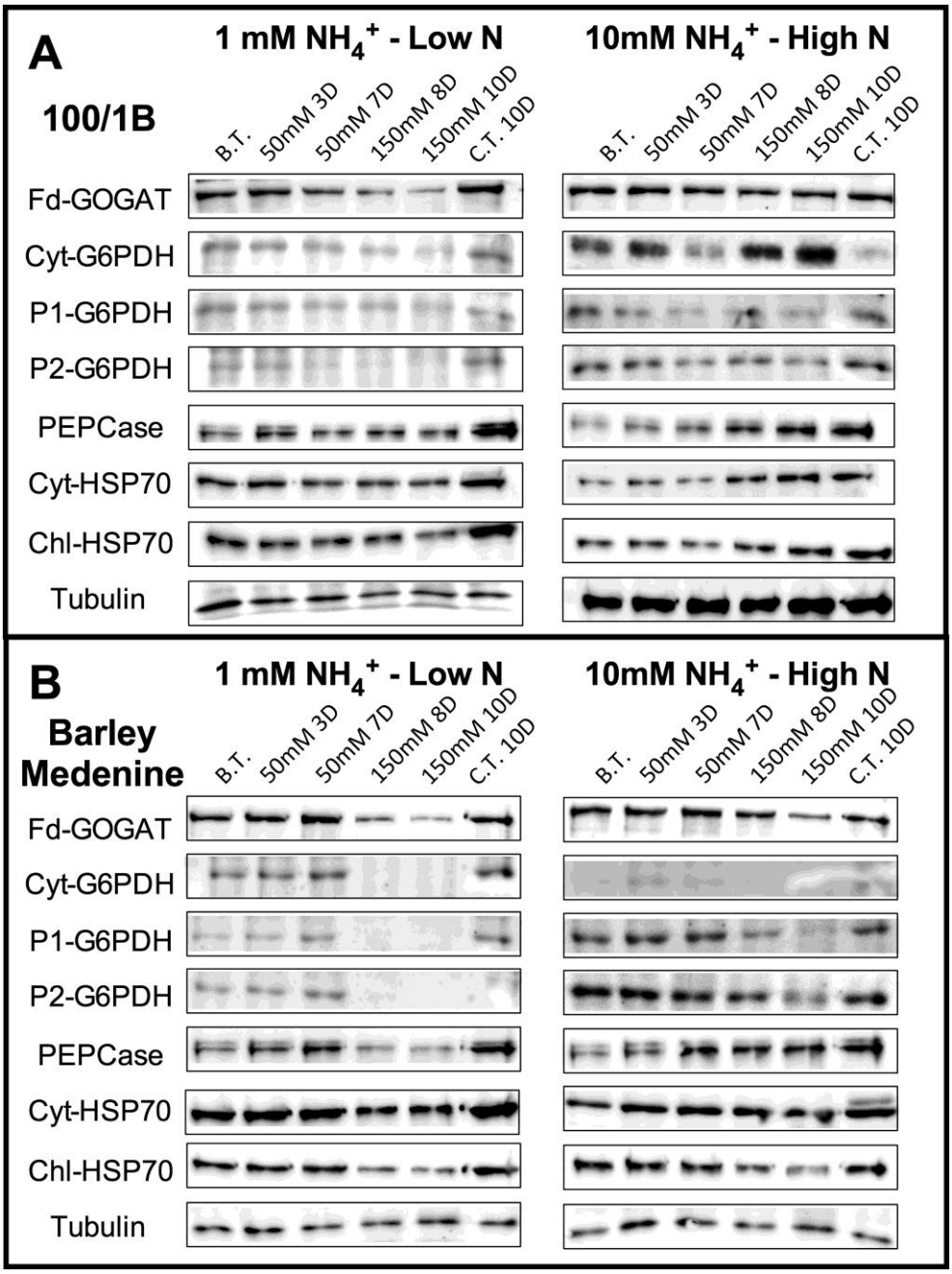

Figure 4: Western blotting of leaves of "100/1B" and "Barley Medenine" grown under low N (1mM NH_4^+) and high N (10 mM NH_4^+) using antisera Fd-GOGAT, cyt-G6PDH, P1-G6PDH, P2-G6PDH, Chl-HSP70, Cyt-HSP70, PEPCase and Tubulin (as control for equal loading). Legend: (BT), Before treatment; (50 mM 3D), 3 days of moderate stress; (50 mM 7D), 7 days of moderate stress; (150 mM 8D), 7 days in 50 mMNaCl and 1 day in 150 mMNaCl (severe stress); (150 mM 10d) 7 days in 50 mMNaCl and 3 days in 150 mMNaCl (severe stress); (CT 10D) control treatment for 10 days. Images are representative of two or three WB from different experiments.

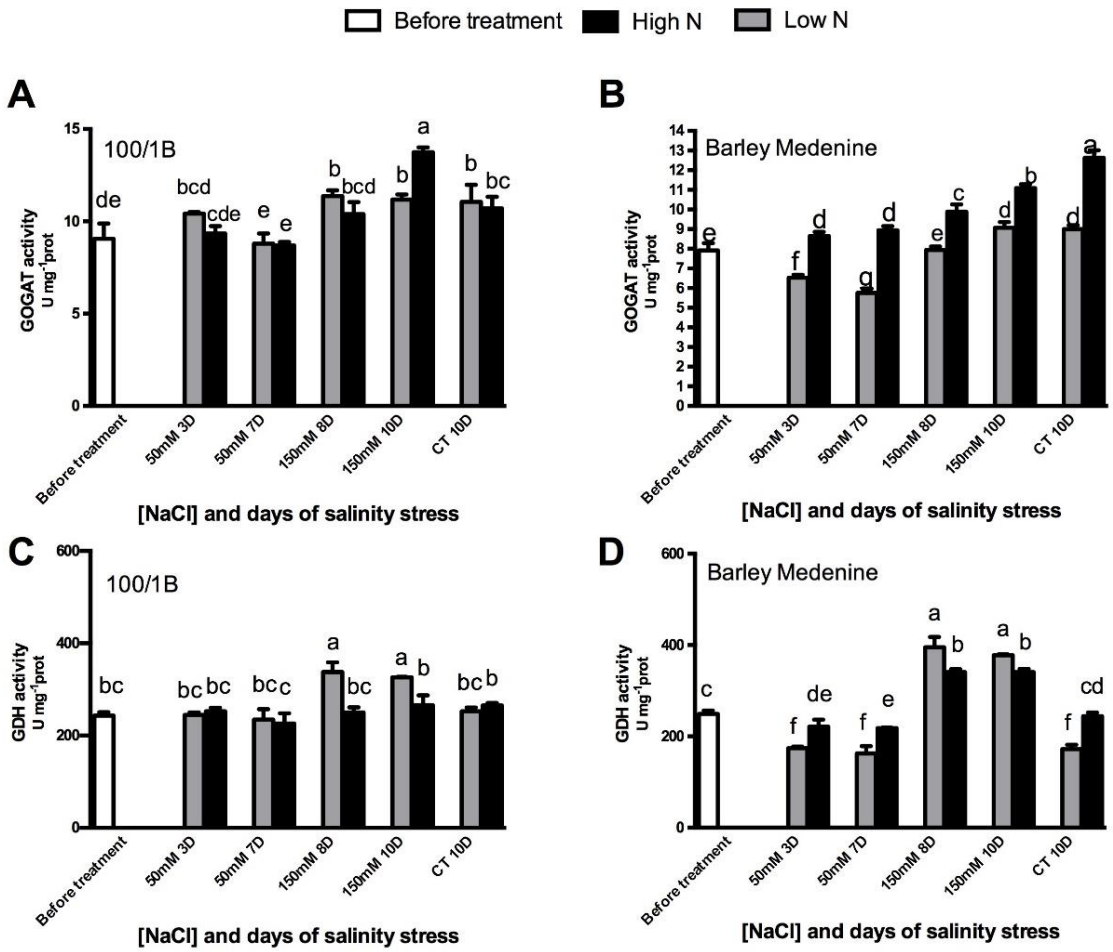

Figure 5: Effects of salinity and N concentration on NADH-GOGAT and GDH enzymatic activities in barley plants growth in hydroponic system. Levels low N concentrations are in grey bars high concentrations are in black bars. Legend: (BT), Before treatment; (50 mM 3D), 3 days of moderate stress; (50 mM 7D), 7 days of moderate stress; (150 mM 8D), 7 days in 50 mMNaCl and 1 day in 150 mMNaCl (severe stress); (150 mM 10d) 7 days in 50 mMNaCl and 3 days in 150 mMNaCl (severe stress); (CT 10D) control treatment for 10 days. Letters indicate significant differencesbetween different treatments. ($P < 0.05$, ANOVA: Duncan test).

4.3. Change in the activity of PEPcase

As shown in Figure 6, the tolerant "100/1B" had an increase in PEPC activity upon N supply and NaCl. In the susceptible "Barley Medenine" , PEPC activity under high N was relatively stable throughout salt stress but lower than control. A 3d-response increasing PEPC activity was observed in low N under both moderate andsevere salinity. Western blotting analysis showed an unchanged PEPC occurrence in low N "100/1B" plants and anincrease in high Nplants under severe salinity (Figure 4). On the contrary, in low N "Barley Medenine" plants,

PEPC occurrence was reduced under high salinity, while a high N supply maintained a relative stability in PEPC abundance, even under severe stress (Figure 4).

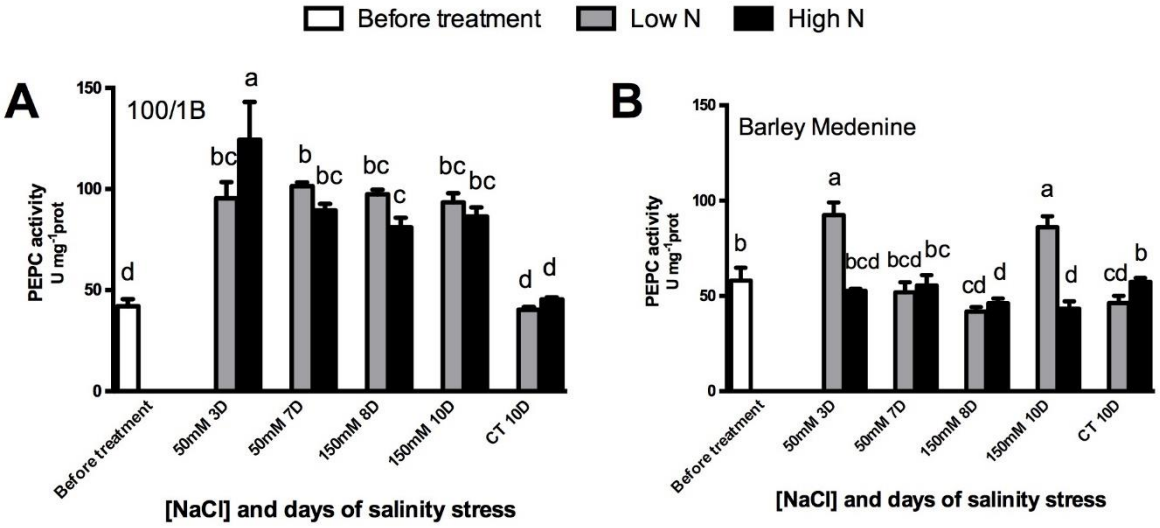

Figure 6: Effects of salinity and N concentration on PEP carboxylase (PEPC) enzymatic activity in barley plants growth in hydroponic system. Legend: (BT), Before treatment; (50 mM 3D), 3 days of moderate stress; (50 mM 7D), 7 days of moderate stress; (150 mM 8D), 7 days in 50 mMNaCl and 1 day in 150 mMNaCl (severe stress); (150 mM 10d) 7 days in 50 mMNaCl and 3 days in 150 mMNaCl (severe stress); (CT 10D) control treatment for 10 days.Levels low N concentrations are in grey bars high concentrations are in black bars. Letters indicate significant differencesbetween different treatments. ($P < 0.05$, ANOVA: Duncan test).

4.4. Correlation between suppliers of reducing power (G6PDH and PEPC) and different enzymes involved in N metabolism or defense against stress

Both G6PDH and PEPC participate, directly or indirectly to N assimilation, by providing NAD(P)H and carbon skeletons supply, respectively; therefore, under stress conditions, a diversion of metabolism to ROS detoxification can be possible. To achieve a deeper understanding of G6PDH and PEPC effects on other enzymes, the correlations between different enzymes havebeen evaluated. In "100/1B" landrace cultivated under 50 mMNaCl, GOGAT was strongly and positively correlated with G6PDH (r= 0.84, p= 0.011) (Table 1). GDH and APX were moderately correlated with PEPC activity (r= 0.54, p=0.176 and r= 0.52, p= 0.195 respectively). Under severe stress, GDH was strongly and positively correlated with both G6PDH and PEPC (r= 0.87, p=0.005 and r= 0.9, p= 0.002 respectively) and inversely correlated with APX (r = -0.85, p= 0.008) (Table 1).On the other hand, "Barley Medenine" showed a positive correlation of GOGAT with G6PDH (r=0.76, p=0.027), and of APX with PEPC (r=0.7, p=0.058) upon severe salinity (Table 2).

Table 1: Coefficients of correlation (r) between different enzymes measured in "100/1B" cultivated under 50 mMNaCl (moderate salt stress – lower diagonal data) or 150 mMNaCl (severe salt stress – upper diagonal data).

"100/1B"	GOGAT	GDH	G6PDH	APX	PEPC
GOGAT		0.53 (0,173)	0.84 (0.011)	-0.28 (0.5)	-0.07 (0.875)
GDH	-0.11(0.799)		0.4 (0.345)	0.21 (0.622)	0.54 (0.176)
G6PDH	-0.35(0.389)	0.87(0.005)		-0.24(0.564)	0.25(0.558)
APX	0.26(0.539)	-0.85(0.008)	-0.97(0.000)		0.52(0.195)
PEPC	0.03(0.942)	0.90(0.002)	0.76(0.030)	-0.78(0.021)	

Table 2: Coefficients of correlation (r) between different enzymes measured in "Barley Medenine" cultivated under 50 mMNaCl (moderate salt stress –lower diagonal data) or 150 mMNaCl (severe salt stress – upper diagonal data).

"Barley Medenine"	GOGAT	GDH	G6PDH	APX	PEPC
GOGAT		0.92(0.001)	0.04(0.916)	0.33(0.430)	-0.35(0.402)
GDH	-0.84(0.009)		0.14(0.737)	0.55(0.155)	-0.35(0.389)
G6PDH	0.76(0.027)	-0.57(0.141)		0.20(0.642)	0.26(0.534)
APX	0.48(0.229)	-0.17(0.684)	0.19(0.645)		-0.39(0.336)
PEPC	-0.16(0.705)	0.23(0.576)	-0.50(0.206)	0.7(0.058)	

5. Discussion

NADH-GOGAT and NADH-GDH regulation were related to N availability in the tolerant "100/1B" which was least affected by salinity compared to the sensitive "Barley Medenine". Tolerant "100/1B", in order to face salinity,stimulates GS/GOGAT pathway under sufficient N nutrition; these results are in accord to previous studies on chick-pea (*Cicer arietinum* L.), soybean (*Glycine max* L.) and halophyte species, (Soussi et *al*., 1998, Stewart and Rhodes, 1978). This stimulation looks triggered to allow the assimilation of the excess of NH_4^+ accumulated under salinity (Nigel et *al*., 2010).

"100/1B" subjected to salinity is able to switch the main GS/GOGAT cycle to GDH assimilation under low N, possibly to limit energy-consuming metabolism. In fact, despite its high efficiency, GS/GOGAT pathway is an energy consuming process when compared to GDH assimilation, by requiring an extra ATP molecule (Nigel et *al*., 2010). Through this mechanism, plants can save energy for the defense system and adaptation to salinity (Hasanuzzaman and Tanveer, 2020). The switching under salinity on the NADH-GDH pathway and inactivating of

GS/GOGAT cycle was previously reported by Ashraf et *al*., (2018). In "Barley Medenine", GOGAT activity significantly decreased upon salinity. Previous studies on different crop had shown that salt stress inhibits the GS/GOGAT cycle, due both salt ions damaging effects or/and substrate limitation (Wang et *al*., 2007, Meng et *al*., 2016, Ullah et *al*., 2019). The reduced GS/GOGAT activity in "Barley Medenine" upon salinity is accompanied by high aminating GDH activity, allowing a direct incorporation of ammonia to glutamate (Wang et *al*., 2014).

The noticeable NADH-GDH increase after 24 h of severe salinity in both genotypes reflects a rapid leaf protein catabolism induced by salt and leading to high intracellular NH_4^+ levels (Ashraf et *al*., 2018). The alternative N assimilation pathway (GDH) activation by salinity is strongly required to protect plant against ammonium toxicity, fill glutamate pool and allow the synthesis of stress-protective metabolites (Goel et *al.*, 2015). Similarly, the GDH activity increase under salinity has been previously reported in Triticale (× *Triticosecale*Wittm.), rice (*Oryza sativa* L.) (Kwinta and Cal 2005; Wang et *al*., 2012). NADH-GDH enzyme presents a low affinity for NH_4^+, and it can be stimulated to synthesize glutamate only when NH_4^+ concentration > 1mM, (Wang et *al*., 2007; Canuto, 2012); so the fact that NADH-GDH was stimulated under low N (1mM NH4+) supports the hypothesis of intracellular ammonia accumulation under salt stress. High levels of antioxidant enzymes are generally considered as a biochemical index of plant tolerance against salinity (Li et *al*., 2009,Guerrier, 1988), but some authors consider scavemgers enzymesas a symptom of stress, suggesting that their increase reflect oxidative damage (Maksimović et *al.*, 2012). Intriguingly, the rapid (1d after salt stress) triggering of defense system in tolerant N-fed "100/1B", by increasing APX and PEPC activities,suggest their role in plant resistance to oxidative damage. In the same context, the increase of APX and PEPC can be considered as a marker of plant tolerance against salinity (Guerrier, 1988; Li et *al.*, 2009). It has been reported that plant species less affected by oxidative stress showed a rapid increase in APX activity in response to stress (Xu et *al*, 2014; Akbari et *al.*, 2020). In contrast, the delayed response of "Barley Medenine" in the increase APX and PEPC activities, observed only 3d after salt stress, and more evident under low N, may suggest that high levels of APX and PEPC activities can be considered as a symptom of oxidative stress in this sensitive genotype. Similarly, Maksimović et *al.*, (2012) proved that APX increase in barley can be a stress sensor. Furthermore, our results confirm the role of N in maintaining metabolic functions under salt stress (Chang et *al.*, 2016)**.** In accordance with our findings, previous studies have shown that different plant responds to salinity by increasing APX and PEPC activities (Sagi et *al*., 1998, Shi et *al*., 2001). Despite APX stimulation only under severe salinity, PEPC was induced even under moderate stress in both genotypes,more evidently in the

tolerant "100/1B". PEPC increase could be due both to the rise in pH caused by the excess of NH_4^+ cations under salinity or to the shortage of oxoglutarate needed in the respiratory cycle and osmoregulation, in order to supply carbon skeletons to TCA cycle and synthetise2-oxoglutarate (Sagi et *al*., 1998, Kant, 2007).

G6PDH plays an important role in sustaining the redox state of plant cells through supplying NADPH reductants needed for ROS-detoxification mechanisms (Esposito, 2016). G6PDH increase in both genotypes reflects the oxidative damage produced by salinity and the high requirement for reductants to triggers defense system against oxidant species.Cy-G6PDH plays an essential role in defense against stress by providing NADPH (Scharte et *al*., 2009) and synthesizing cofactors involved in the tolerance mechanisms (Dal Santo et *al*., 2012).Therefore, the higher occurrence of cyt-G6PDH in "100/1B" in response to salinity and N availability can justify the tolerance of this genotype. In agreement with our results, a previous study showed a significant increase in the expression and abundance of cytosolic G6PDH upon abiotic stress (Landi et *al*., 2016). Heat shock proteins (HSP70s) are crucial regulators of response against abiotic stress (Landi et *al*., 2019). The maintain or the increase of HSP70s isoforms and PEPC occurrence in "100/1B" and its decrease in "Barley Medenine" in response to salinity could explain the tolerance of the first and susceptibility of the second.

The participation of both G6PDH and PEPC in the NADPH recycling machinery may indicates a correlation between different enzymes, in its turn depicting different defense scenarios of the two genotypes upon salinity. Under moderate stress, the positive correlation between G6PDH and GOGAT suggests that the reductants produced via G6PDH can be used in N metabolism in the GS/GOGAT cycle. The positive correlation of PEPC with both APX and GDH indicates the PEPC involvement in both ROS scavenging system and N assimilation via GDH pathway. Under severe salinity, based on the strong correlation between GDH with both G6PDH and PEPC, we suggest that severe salinity leads to a diversion of reductants supplied by G6PDH and PEPC to the alternative N assimilation pathway (GDH).Based on the observed correlation under high salinity in the sensitive "Barley Medenine", we suggest that the involvement of G6PDH in N assimilation via GS/GOGAT cycle and the participation of PEPC (Esposito et *al*., 1998) in response against salinity as well.

Triggering a defense system, while maintaining an efficient nitrogen metabolism, is one of the keys of plant tolerance against abiotic stress. Our findings proved that the tolerance of "100/1B" and the sensibility of "Barley Medenine", which have been previously selected based on agronomic parameters, is based on evident metabolic changes. The results here presented support the hypothesis that activities and occurrence of specific enzymes can be used as a useful

index of stress tolerance in specific genotypes. However, further physiological, biochemical and molecular studies are required to better understand plant regulation system under stressful circumstances

General Discussion and Conclusion

In Tunisia, the water deficit, the poor quality of irrigation water, are the most limiting factors for cereals production, particularly in arid and semi-arid areas. The valorization of these areas in agriculture is faced with other difficulties, such the salt tolerance degree of cultivated species. Barley (*Hordeum vulgare* L.) is one of the cereals tolerant to salt stress and arid climates. Nitrogen (N) is one of the most important macronutrient that can improve crop yield and alleviate the adverse effects salinity on crop (Murtaza et *al*., 2013) if it is well managed.

The study of the interaction between salinity tolerance and Nitrogen metabolism in barley genotypes was investigated through multi-year and multi-treatment trials under both field and controlled conditions. Results in chapter I, II, III and IV indicated that N addition had a positive and corrective effect on grain and biomass yield, morphological, physiological and biochemical traits for all tested genotypes.

In Chapter I, grain and biomass yield, N uptake, and grain protein content showed a significant increase with increasing N rates, and significant decrease when plants irrigated with saline water. Average yield for all genotypes ranged from 2.4 t/ha without N supply to 4,8 t/ha for the fertilized plants under low saline irrigation; while it was It was varied between 1 and 4.5 t/ha under high salinity conditions. It appears from our results that under low saline irrigation increasing N fertilization generally increased yield particularly below 150 kgN/ha, and that between 100 and 150 kgN/ha there was a decrease or no significant enhancement of production. While in saline conditions, 150 KgN/ha application improved grain yield only for the two landraces "100/1B" and "Barley Medenine". These results confirm that optimum N rate required to achieve the maximum grain yield in arid region is estimated to be 100 kg N/ha under low saline irrigation, 150 kgN/ha or 100 KgN/ha under saline irrigation depending on genotypes. Therefore, adding N beyond 100KgN/ha decreased grain yield in "100/1B" and the improved "ICARDA20" may due to nutrient toxicity or lodging effect (Godard et *al.*, 2008). Similar results indicated that in mediterranean environment wheat grain yield increased with N supply up to 100 kg N ha^{-1}, then grain number and yield decreased with high N fertilization (Abad et *al*., 2004, wang et *al*., 2011).

Grain yield was mostly enhanced through the increase of grain number m^{-2} which in turn was strongly correlated with N uptake and biomass production. The decrease of grain yield under saline irrigation is mainly related to the negative effect of saline water on total N uptake which was severely reduced probably because of the decrease of biomass production. Biomass yield increased with the increase of N up to the highest rate of 100 or 150 kg/ha depending on genotypes. In fact, under low saline irrigation the two tolerant genotypes "100/1B" and "Souihli" reach a maximum yield under 100KgN/ha. The tolerant landrace "100/1B" was the

most stable and the less affected by salinity: 1.8 t/ha of biomass yield reduction under saline irrigation compared to 2.35 T/ha in the other genotypes. Interestingly, whatever saline and nutritional conditions, "Souihli" produced higher biomass than other genotypes while "Barley Medenine" recorded the lowest biomass yield and the least beneficiary from nitrogen. "Souihli" was the most able to absorb N under all saline and nutritional condition at both field and controlled assay**.** The low field productivity of "Barley Medenine" can be explained by the high proportion of awns production to biomass and spike weight, and the alteration of N allocation pattern to the spike (high N accumulation in "Barley Medenine" awns associated with the low grain N accumulation). In fact, awns development known to compete ovary growth for assimilate, and then influence grain yield because of the evident relation between grain number with ovary size (Guo et *al*., 2015; Xie et *al.,* 2015). Rebetzke et *al*. (2016) report that allocation of assimilates to developed awns reduce grain number and increase sterile spikelets number leading to reduce grain yield. Our results suggest that under 150N, an amount of absorbed N was not remobilized to grain but remained in straw or allocated to awns. In fact, under 150N treatment the improved "ICARDA20" accumulate 42% of the total N uptake in straw compared with 27% under sub 150N doses; whereas the landrace "100/1B" accumulate 13,5% of the total N uptake in awns compared with 8.5% under sub 150N doses. In this context, previous study reported that high N nutrition increased stomatal conductance, which in turn increased the loss of N from the top of plants (Harper et *al*. 1987; Farquhar et *al*. 1980).

The response of barley genotypes showed that N fertilization was more evident for productivity than grain quality. In fact, N allocation to sinks firstly improved grain number rather than protein content; only under plentiful N condition (low salinity) are both grain number and protein content maximum achieved **(**Zhang et *al.*, 2015; Perchilik and Tegeder, 2018), whereas under salinity N absorption was altered. Barley productivity in arid environments was influenced by annual rainfall, results showed that the important rainfall amount during stem elongation (the most active developing period of crops) in the second year enhanced markedly GY of salt stressed plants mostly through leaching of salts from the root zone. On the other hand, as showed in the chapter II, rainfall can causes N losses and affect the N uptake especially under low saline irrigation.

Salinity and N fertilizer significantly influenced NUE and its components (chapter II, III). Under our both experimental conditions, NUE decreased with increasing N rates, this decrease was 21% under field conditions whereas it reach 80% in controlled assay. Several reports, such as Perchilik and Tegeder (2017) showed that N fertilizer addition is the main reason for low N efficiency. Similarly with some other studies our results proved that salinity disrupted NUE

through the reduction of N uptake and N partitioning patterns (Ciampitti and Vyn, 2013; de Oliveira Silva et *al*., 2017). 28.5% of reduction was caused by salinity stress in the field experiment. "ICARDA20" and "Souihli" landrace displayed the maximum average of NUE (25.5 Kg/KgN) under field conditions, while they were "100/1B" and "Souihli" the most efficient under controlled assay (23mgDW/mgN and 19 mgDW/mgN respectively). Several authors showed that plant response to salinity-nitrogen interaction in controlled conditions are mostly distinct from those of field because of the complexity of the natural conditions (Ashraf et *al*., 2018).

In Chapter II and III, NupE and NutE as dependent NUE components showed different trends according to salinity and N fertilization levels. As expected salinity decreased the total amount of absorbed N by 41% and 26% in field and controlled conditions respectively. On the other hand N fertilizer alleviates the detrimental effect of salinity on total N uptake and N accumulation in grain, straw and awns. Compared results has been shown in wheat and rice (Guo et *al*.,2016; Wang et *al*., 2011; Barraclough et *al*., 2010). The luxury N accumulation observed in "Souihli" which continued to take up N fertilizer under 150N despite the reaching of the maximum grain and biomass yield under 100N can explain the tolerance and the important grain weight and quality of Souhili. Our results suggest that luxury N accumulation not used for grain and biomass yield but incorporated into "N reserve pool" to buffer against stresses or/and improve grain qualities. The findings that in both experiment (field and controlled conditions) "Souihli" efficiently absorb and allocate N to grain (in field assay) or shoots (greenhouse assay), together with the possession of the highest grain N concentration, grain N accumulation, grain weight, grain yield, grain protein yield and N concentration in shoot and roots in the greenhouse assay support the hypothesis of the luxury N accumulation and "N reserve pool. Furthermore "Souihli" was the only genotypes that showed a positive relationship between GY and GPC in both saline conditions which reinforce the hypothesis of "N reserve pool". Lopez-Bellido et *al*., (2004) approved that at certain level of N supply both yield and GPC increase with increasing N fertilizer only when N uptake is high (Barraclough et al., 2010) which was proven in "Souihli".

It is important to underline that "ICARDA20" showed a high N uptake under favorable conditions (N application and low saline irrigation), but it exhibited an important decrease under salinity or N deficiency under both assay. Interestingly, the two genotypes "ICARDA20" and "Souihli" were the most enhanced by N addition to increase N uptake in both assay (field and greenhouse assay).

Our results showed that salinity affected negatively NUpE and caused a decrease of 40.3% under field conditions and 24% in greenhouse assay. In both assay the lowest value of NupE was observed in "Barley Medenine" (48% in field and 15% in greenhouse) and the highest NUpE was observed in "Souihli" (65.3% in field and 22.9% in green house). NupE showed a significant decrease with N supplying: 55% and 17.3% were the general reduction observed in controlled and field assay respectively. Similar results were reported by Moll et *al*. (1982), Sinebo et *al*. (2004) and Perchilik and Tegeder (2017) indicated that NupE was higher at low N rates and decreased with increasing N application. Our findings showed that genotypic variability between the four genotypes was due to difference in NupE or NutE. In fact, the two tolerant genotypes "100/1B" and "Souihli" showed a great ability to absorb N from the soil when it is limited, which is of greater importance to enhance NupE, NUE and crop productivity. Similarly, Le Gouis et *al.* (2000) found that NupE accounted for most of the variation in NUE as compared to NutE at low N supply. Under sufficient N condition, N will be accessible to plant independently of the efficiency of N absorption and root system; in that case NutE will be of greater importance to determine and enhance NUE. This controversy may be partially due to the soil texture, its capacity of N fixation and to the genetic variability related to root architecture (Sanguineti et *al*., 2007). Under field conditions there was no important change in NUtE with increasing N rate, it deccreased slightly (7.8%) only under high N fertilization (150KgN/ha). Cox et *al*. (1986) revealed that high N fertilisation before wheat flowering led to decrease N remobilization because it increased post-anthesis N uptake and make N remobilisation less necessary, whereas low N treatments lead to increase N remobilisation (Barbottin et *al*., 2005), which explain the increase of NUtE by 19% at both assay in all genotypes under salinity except "Souihli" which whowed unchanged NutE value in controlled assay. Interestingly the improved "ICARDA20" showed the highest NutE (45,75 kg grain/kg uptaken N). Our finding showed that NUtE was positively correlated with GY only in the landrace "100/1B" and the improved "ICARDA20" grown under salinity: 33% and 45% of GY variation under salinity was only explained by NutE in "100/1B" and "ICARDA20" respectively.

Overall morphologic and physiologic traits, and NUE components differed between genotypes in confirming that there is a genetic variability in biochemical traits such as specific enzymatic activities and protein occurrence. At seedling stage, the most stable genotype under salinity ("100/1B") and the most sensitive ("Barley Medenine") grown in hydroculture showed a great genetic variation. "100/1B" and "Barley Medenine" differed in their response to NaCl addition, in fact the tolerant "100/1B" was least affected by salinity compared to the sensitive "Barley

Medenine". In order to face salt stress and allow the assimilation of the excess of NH_4^+ accumulated under salinity, the response of "100/1B" genotype was depending on N availability: "100/1B" stimulates GS/GOGAT pathway under sufficient N nutrition, and switch the GS/GOGAT cycle to GDH assimilation under low N. The switching of the GS/GOGAT cycle to GDH pathway under salinity and low N -possibly to reduce energy consuming- can be a mechanism of plant tolerance to salinity and N deficiency, and may explain the stability of "100/1B" under all unfavorable conditions. Through this mechanism, plants can save energy for the defense system and adaptation to salinity (Hasanuzzaman and Tanveer, 2020). Similar results of activating NADH-GDH pathway and inactivating GS/GOGAT cycle under salinity was previously reported by Ashraf et *al*., (2018). The sensitive "Barley Medenine" genotypes showed a significant decrease in GOGAT activity under salinity which reflect the inhibition of the GS/GOGAT cycle, this reduction is accompanied by high aminating GDH activity, allowing a direct incorporation of ammonia to glutamate (Wang et *al*., 2014). Severe salt stress increased noticeably NADH-GDH in both genotypes which suggest a rapid leaf protein catabolism induced by salt and leading to high intracellular NH_4^+ levels (Ashraf et *al*., 2018), in that case GDH pathway activation is strongly required to protect plant against ammonium toxicity, fill glutamate pool and allow the synthesis of stress-protective metabolites (Goel et *al.*, 2015).

"100/1B" showed a rapid triggering of defense system by increasing APX and PEPC activities (after 1d), which suggest their role in plant resistance to oxidative damage. At the opposite the sensitive genotype "Barley Medenine" showed a delayed response (3d) to increase APX and PEPC activities, which was more evident under low N treatment. Taken together, these results suggest that the rapid increase of APX and PEPC in "100/1B" can be considered as a marker of plant tolerance against salinity (Guerrier, 1988; Li et *al.*, 2009). Similar results reported that plant species less affected by oxidative stress showed a rapid increase in APX activity in response to stress (Xu et *al*, 2014; Akbari et *al.*, 2020). Whereas the delayed response to increase APX and PEPC activities under severe stress and N deficiency can be a symptom of oxidative stress in sensitive "Barley Medenine". In the same context, Maksimović et *al.*, (2012) indicated that APX increase in barley plants can be a stress sensor. Our results confirm the role of N in maintaining metabolic functions under salt stress (Chang et *al.*, 2016)**.** As known, Cy-G6PDH plays a key role in defense against stress by providing NADPH (Scharte et *al*., 2009) and synthesizing cofactors involved in the tolerance mechanisms (Dal Santo et *al*., 2012), and also Heat shock proteins (HSP70s) are crucial regulators of response against abiotic stress (Landi et *al.*, 2019). Therefore, the higher occurrence of cyt-G6PDH in the tolerant "100/1B" in response to salinity and N availability, and the maintain or the increase of HSP70s isoforms

and PEPC occurrence in "100/1B" can justify the tolerance of this genotype. On the other side the decrease of HSP70s isoforms and PEPC occurrence in "Barley Medenine" could explain its susceptibility.

Our works showed the suitability of the Mediterranean arid climate for barley production by the application of optimal N fertilization which could alleviate the negative effect of salinity. Based on this study it is generally concluded that supplying N fertilizer at the rate of 100 $kgN.ha^{-1}$ could be recommended to avoid N losses and achieve grain yield performance, and at the rate of 150 $kgN.ha^{-1}$ to achieve Biomass yield performance.

Maintaining an efficient N uptake and utilization under stressful circumstances, and activating detoxification mechanisms are the keys to obtain high performance. NUE, N uptake and distribution pattern, K^+ uptake, the stabilization of the photosynthetic rate to maintain growth, and activities and occurrence of specific enzymes can be a precious tool to predict the tolerance or the susceptibility of barley genotypes. In addition, luxury accumulation of the excessive N and its incorporation into "N reserve pool" , with the efficient use of N are researched phenomenon to enhance salt tolerance and contribute to a high grain quality. Our results showed a great genotypic variability within the used genotypes. The stability of "100/1B" genotype under all unfavorables conditions could be attributed to its ability to regulate metabolic functions under salinity and/or N deficiency, and its high N uptake under low N fertilization. The performance of "Souihli" genotype is mainly due to its capacity to absorb efficiently N and to incorporate it into "N reserve pool" to buffer against stresses and enhance grain quality. "ICARDA20" genotype showed a high performance especially in the absorption and utilization of N, but only under specific favorable conditions. These genotypes presented an interesting genetic potential that could be exploited as a reservoir of genetic flexibility to improve tolerance to salinity and plant NUE in future work. The present study established the interaction between salinity and nitrogen metabolism on barley. Although the number of genotypes is limited, this work suggested a number of useful index of salinity tolerance. This has to be more investigated through the use of a larger number of genotypes. Isotopic labeling and molecular studies may provide further insights into nitrogen metabolism under salinity, luxury N reserve mechanism and plant regulation system under abiotic stress. For a sustainable agriculture, a long-term soil productivity study should be included in future work.

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Publications