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Effect of sodium chloride on the germination of carob *(Ceratonia siliqua L.)* seeds

Presented by: :

- M_{elle} : Moussaoui Hadil
- M_{elle} :Ouis Soumia

Defended on:

Before the examination committee composed of :

President	Dr.ZOUAOUI khadidja	MCB, UMTS University of Saida
Examiner	Dr. BERBER Naima	MCA, UMTS University of Saida
Rapporteur (Supervisor)	Pr. CHALANE Fatiha	Pr, UMTS University of Saida

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I dedicate this work with great love and gratitude,

To my dear parents,

Words cannot express my deep appreciation and endless gratitude for your unconditional support, sacrifices, and encouragement throughout my life. This work is the result of your prayers and efforts.

To my brothers and sisters,

For their continuous support and encouragement.

To my family,

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To my friends,

Who stood by me during both difficult and joyful moments.

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Dedication

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Soumia

Abstract of the Thesis

This study investigates the effect of salinity stress on the germination of *Ceratonia siliqua L* seeds, a Mediterranean species known for its ecological and socio-economic importance and its tolerance to harsh environmental conditions. Soil and water salinity represent a major abiotic stress factor limiting plant growth and agricultural productivity, particularly in arid and semi-arid regions such as Algeria. Understanding the response of *Ceratonia siliqua L* seeds during the germination stage is essential for improving its propagation and use in reforestation and land restoration programs.

Temperature is a key environmental factor influencing seed germination by regulating metabolic and enzymatic activities. In this study, exposure to low temperature (5°C) resulted in complete inhibition of germination, confirming the high sensitivity of *Ceratonia silique L* seeds to cold stress.

The experimental work was conducted under controlled laboratory conditions using *Ceratonia siliqua* seeds subjected to different treatments, including dormancy-breaking methods (mechanical scarification, thermal and cold treatments), disinfection, and exposure to varying concentrations of sodium chloride (NaCl: 0, 100, and 200 mM), as well as a cold stress condition at 5°C. Germination parameters such as germination percentage, germination rate, radicle length, and germination vigor were evaluated over a 21-day period.

The results showed that germination was highest under control conditions (0 mM NaCl) and decreased significantly with increasing salinity levels. Moderate salinity (100 mM NaCl) reduced germination and seedling growth, while high salinity (200 mM NaCl) severely inhibited germination and radicle elongation. Complete inhibition of germination was observed under cold stress (5°C). Among the dormancy-breaking treatments, mechanical scarification was the most effective in enhancing germination by improving water uptake through the hard seed coat.

Overall, the findings demonstrate that *Ceratonia siliqua* seeds are highly sensitive to salinity and low temperature during germination, despite the species' known drought tolerance at later developmental stages. This study contributes to a better understanding of the physiological responses of *Ceratonia siliqua* seeds under environmental stress and provides useful insights for seed propagation, reforestation strategies, and the management of saline soils in Mediterranean and arid regions.

Keywords *Ceratonia siliqua,* , germination, seed physiology, drought-adapted species, , dormancy breaking.

Résumé

Cette étude a pour objectif d'évaluer l'effet de différentes concentrations de chlorure de sodium (NaCl) sur la germination des graines de *Ceratonia siliqua L.* ainsi que sur le développement précoce des plantules, notamment la croissance de la radicule. L'influence du stress thermique (5 °C) et de différents traitements de levée de dormance a également été étudiée.

La température est un facteur environnemental clé influençant la germination des graines en régulant les activités métaboliques et enzymatiques. Dans cette étude, l'exposition à une basse température (5 °C) a entraîné une inhibition complète de la germination, confirmant la forte sensibilité des graines de caroubier au stress thermique froid.

Les résultats montrent que la germination des graines de *Ceratonia siliqua.L* est fortement influencée par les conditions environnementales. Les meilleures performances ont été obtenues dans les conditions optimales (0 mM NaCl et 27–28 °C), avec un taux de germination élevé et une croissance normale de la radicule. En revanche, l'augmentation des concentrations en NaCl a entraîné une diminution progressive de la germination et de la croissance racinaire, jusqu'à une inhibition quasi totale à 200 mM NaCl.

Le stress salin agit principalement par effet osmotique et toxicité ionique, limitant l'absorption de l'eau et perturbant les processus physiologiques essentiels à la germination. De plus, le stress thermique (5 °C) a totalement inhibé la germination, indiquant la sensibilité des graines aux basses températures.

Par ailleurs, la scarification mécanique s'est révélée la méthode la plus efficace pour améliorer la germination, contrairement aux traitements thermiques et à la stratification froide.

Cette étude met en évidence l'importance des facteurs abiotiques, notamment la salinité et la température, sur la germination de *Ceratonia siliqua L.*

Mots-clés

Ceratonia siliqua L. ; germination; stress osmotique ; stress thermique ; levée de dormance.

الملخص

تهدف هذه الدراسة إلى تقييم تأثير تراكيز مختلفة من كلوريد (NaCl) على إنبات بذور شجرة الخروب (*Ceratonia siliqua L.*) الصوديوم

بالإضافة إلى دراسة النمو المبكر للشتلات، خاصة طول الجذير. كما تم دراسة تأثير الإجهاد الحراري (درجة حرارة (5°C) وبعض معاملات كسر السكون على عملية الإنبات منخفظة

تُعدّ درجة الحرارة عاملاً بيئيًا أساسيًا يؤثر في إنبات البذور من خلال تنظيم الأنشطة الأيضية والإنزيمية. في هذه الدراسة، أدى التعرض لدرجة حرارة منخفضة (5°م) إلى تثبيط كامل لعملية الإنبات، مما يؤكد الحساسية العالية لبذور الخروب للإجهاد الحراري البارد

أظهرت النتائج أن إنبات بذور الخروب يتأثر بشكل واضح بالظروف البيئية. فقد سجلت أفضل النتائج في الظروف المثلى

ودرجة حرارة (28–27°C) حيث تم الحصول على نسبة إنبات مرتفعة ونمو طبيعي للجذير. في المقابل، أدى ارتفاع تركيز

(0 mM NaCl)

الملوحة إلى انخفاض تدريجي في نسبة الإنبات وضعف في النمو، مع تثبيط شبه كامل عند (200 mM NaCl)

ويرجع تأثير الملوحة أساساً إلى الضغط الأسموزي الذي يقلل من امتصاص الماء خلال مرحلة الامتصاص، بالإضافة إلى السمية الأيونية عن تراكم أيونات الصوديوم والكلور، مما يعيق العمليات الفسيولوجية الضرورية للإنبات. كما أدى (5°C)الناتجة إلى توقف كامل انخفاض درجة الحرارة لعملية الإنبات

أما فيما يخص معاملات كسر السكون، فقد تبين أن الخدش الميكانيكي هو الأكثر فعالية في تحسين الإنبات مقارنة بالمعاملات الحرارية والتبريدية.

توضح هذه الدراسة أن إنبات*(Ceratonia siliqua L)* يتأثر بشكل أساسي بعامل الملوحة ودرجة الحرارة، إضافة إلى أهمية

كسر السكون في تحسين نسبة الإنبات بشكل عام .

الكلمات المفتاحية

شجرة الخروب؛ الإنبات؛ الملوحة ؛ الإجهاد الأسموزي؛ الإجهاد الحراري ؛ كسر السكون.

TABLE OF CONTENTS

List of Abbreviations

- **C. siliqua**: *Ceratonia siliqua* L.
- **NaCl**: Sodium Chloride
- **mM**: Millimolar
- **GP**: Germination Percentage
- **ISTA**: International Seed Testing Association
- **ANOVA**: Analysis of Variance
- **PEG**: Polyethylene Glycol
- **SD**: Standard Deviation
- **Caesalpinioideae**: Subfamily of Fabaceae
- **Fabaceae**: Legume family (also called Leguminosae)
- **°C**: Degrees Celsius
- **cm**: Centimeter
- **mg**: Milligram
- **g**: Gram
- **mL**: Milliliter
- **ha**: Hectare
- **K^+**: Potassium ion
- **Na^+**: Sodium ion
- **Cl^-**: Chloride ion

LIST OF TABLE

LIST OF FIGURES

General Introduction

General Introduction

Soil and water salinity represent one of the major environmental problems affecting agricultural productivity worldwide, particularly in arid and semi-arid regions. Salinity is considered a dominant abiotic stress that limits plant growth, development, and yield due to land degradation and improper water management practices (Flowers *et al*., 2010; Munns & Tester, 2008). Current estimates indicate that more than 6% of the world's total land area is affected by salinity, with a continuous expansion of saline soils threatening food security and ecosystem stability (Zörb *et al*., 2019).

In the Mediterranean basin, saline soils cover approximately 16 million hectares. Algeria is among the most affected countries, with nearly 3.2 million hectares of saline land, mainly concentrated in arid and semi-arid regions where salinity constitutes a major constraint to agricultural development (Benzahra & Snoussi, 2018; Benmahioul *et al*., 2009). This phenomenon is often exacerbated by prolonged drought periods, which intensify soil salinization processes and accelerate desertification, leading to ecosystem instability and vegetation degradation (Rengasamy, 2010).

Seed germination is one of the most critical and sensitive stages in the plant life cycle, as it determines successful seedling establishment and plant survival. This stage is strongly influenced by environmental factors such as temperature, water availability, oxygen supply, and salinity (Pearson *et al*., *1997*). Under saline conditions, high concentrations of soluble salts, particularly sodium chloride (NaCl), reduce seed water uptake due to osmotic stress, disrupt ionic balance, and induce toxicity effects that negatively affect germination percentage, speed, and uniformity (Debez *et al*., *2001*; Khan & Gulzar, *2003*).

The *Ceratonia siliqua L* is a perennial Mediterranean species belonging to the *Fabaceae* family. It is highly adapted to harsh environmental conditions such as drought, poor soils, and high temperatures. Due to its ecological resilience and economic importance, it is widely used in reforestation programs, soil conservation, and agro-industrial applications.

Despite its known tolerance to drought and poor soils, the response of *Ceratonia siliqua L* to salinity stress, particularly during the early stages of development such as seed germination, remains insufficiently documented. Although some studies suggest that *Ceratonia siliqua* exhibits moderate tolerance to salinity, its physiological and morphological responses under

increasing NaCl concentrations are not yet fully understood (Ben Naceur et al., 2001; Sbay & Abourouh, 2006).

In Algeria, the cultivation of *Ceratonia siliqua L* has significantly declined over past decades, although recent economic and industrial interest has renewed attention toward this species. This highlights the need for further scientific investigations to improve its establishment and productivity under stressful environmental conditions.

Problem Statement

The main problem addressed in this study is the lack of sufficient information regarding the effect of salinity stress on the germination and early growth of *Ceratonia siliqua L* seeds, particularly under controlled laboratory conditions. Understanding the tolerance limits of this species during germination is essential for improving its propagation and supporting reforestation programs in saline and arid environments.

Hypothèses

- Increasing NaCl concentration reduces germination rate
- *Ceratonia siliqua* seeds show moderate tolerance to salinity
- Temperature and cold stress affect the germination of *Ceratonia siliqua L*. seeds

Objectives of the Study

The main objective of this study is to evaluate the effect of different sodium chloride (NaCl) concentrations on the germination behavior of *Ceratonia siliqua* seeds.

More specifically, this work aims to:

- Determine the effect of salinity (NaCl) on seed germination percentage
- The factors affecting the system
- Analyze the impact of salt stress on germination speed and germination dynamics
- Evaluate the effect of salinity on early seedling growth, particularly radicle length
- you referred to NaCl (sodium chloride)

- Identify the tolerance limits of *Ceratonia siliqua* seeds to NaCl stress during germination
- Compare the effects of salinity and low temperature (cold stress) on germination performance
- Contribute to a better understanding of the physiological responses of *ceratonia siliqua*
- seeds under abiotic stress conditions

Chapter I

Germination and Salinity Stress

I.1 Definition of Germination

Germination is a fundamental biological process that marks the transition of a seed from a dormant state to an active, growing organism. It represents the first critical stage in the plant life cycle and determines the successful establishment of the plant.

Germination is defined as the process by which a seed passes from a latent (dormant) life to an active life under the action of favorable factors (Roger, 2007). This transition begins with water absorption and ends with the elongation of the hypocotyl (Hopkins, 2003).

In other words, germination is the phenomenon by which the embryo resumes growth by mobilizing and utilizing the stored reserves contained within the seed.

However, physiologists define germination more strictly. Germination begins with seed imbibition (water uptake) and ends at the moment when the radicle (embryonic root) emerges from the seed coat (Evenari, 1957). From this physiological perspective, germination is limited to the metabolic reactivation and initial visible growth of the embryo, specifically the protrusion of the radicle (Heller, 2000),

I.2.Germination Stages

Germination occurs through three successive and interdependent phases:

I.2.1 Phase I: Imbibition Phase

The imbibition phase is defined as a rapid and passive water uptake phenomenon.
This stage involves a massive rehydration of previously desiccated cellular structures. This phase is accompanied by a marked increase in respiration (Raven *et al.,* 2003; Meyer *et al.,* 2004).
During imbibition:

- Macromolecules regain their functional conformation.
- Cellular membranes reorganize structurally.
- Metabolic activities begin to restart.

Although this phase is largely physical (water absorption driven by matric forces), it initiates the metabolic reactivation necessary for the following stages.

I.2.2 Phase II: Germination Phase (Lag Phase)

Also referred to as the germination period, this phase is characterized by stable hydration and stable respiratory activity.

After the initial rapid water uptake, absorption continues but at a slower and more regulated rate. This stage is marked by:

General activation of seed metabolism (Hopkins, 2003).

Reversible dehydration capacity without significant loss of viability (Heller *et al.,* 2000).

This reversibility indicates that the seed has not yet reached irreversible growth but is metabolically active.

Metabolic Activation

During this phase:

- Water circulates within the seed.
- Hydrosoluble plant hormones stored in the seed are activated.
- The role of gibberellins includes the following:
- They are transported to the aleurone layer.
- They activate the synthesis of hydrolytic enzymes such as: Alpha-amylases, Nucleases and Proteases. These enzymes are involved in: mobilization of reserve substances, cell division and cellular elongation necessary for embryonic axis development (Anzara, 2006).

This phase is therefore primarily biochemical, involving enzymatic breakdown of stored reserves and preparation for visible growth.

I.2.3Phase III: Growth Phase

The third phase corresponds to the growth of the radicle followed by the tigelle (embryonic stem).

This phase is characterized by:

- Increased respiration
- Continued water uptake
- Active cellular elongation

Unlike the previous phases, this stage represents irreversible growth. The radicle emerges first, marking the visible completion of germination from a physiological perspective.

This phase involves:

- Active growth processes
- Structural expansion of embryonic tissues
- Establishment of the seedling axis

I.3 Factors Affecting Germination

I.3.1Internal Factors

Internal factors are inherent to the seed itself and determine its physiological readiness to germinate.

a) Maturity

A seed is considered mature when all its constitutive parts are completely differentiated both morphologically and physiologically (Chaussant et Deunff, 1975; Heller *et al.,* 2004).

Morphological differentiation implies the complete structural formation of embryonic tissues and seed components. Physiological differentiation refers to the functional readiness of the embryo and storage tissues.

Only fully mature seeds possess the structural integrity and biochemical capacity required to initiate germination under favorable conditions.

b) Longevity

Longevity is defined as the duration during which seeds remain alive and capable of maintaining their germinative power to produce viable seedlings (Vallad, 2002).

This parameter determines the temporal window during which a seed retains its viability. Even if environmental conditions are optimal, germination cannot occur if the seed has lost its vitality due to aging or deterioration.

I.3.2 External Factors

External factors correspond to environmental conditions that directly influence metabolic reactivation and embryonic growth.

a) Oxygen

A low quantity of oxygen may be sufficient to allow germination.

Oxygen availability is controlled by the seed envelopes, which function both as a barrier and as a reserve (Meyer et *al*., 2004).

Oxygen is essential for respiration, which provides the energy required for metabolic processes during germination. The permeability of seed coats therefore plays a decisive role in regulating oxygen diffusion toward the embryo.

b) Light

Light acts differently depending on the species.

It inhibits germination in seeds with negative photosensitivity and stimulates germination in seeds with positive photosensitivity (Anzala, 2006).

This indicates that germination response to light is species-specific. Some seeds require exposure to light as a stimulatory signal, whereas others germinate more effectively in darkness.

c) Temperature

Temperature plays a role in increasing the speed of chemical reactions that stimulate germination (Domonique, 2007).

Additionally, it favors the solubility of oxygen within the embryo (Chaussant, 1975). By accelerating metabolic reactions and improving oxygen availability, temperature directly influences the rate and efficiency of germination processes.

- **Effect of Low Temperature (Cold Stress) on Seed Germination**

Temperature is one of the most critical environmental determinants regulating seed germination. Germination occurs within defined cardinal temperatures: minimum (base), optimum, and maximum. When temperature approaches the lower cardinal threshold, metabolic and developmental processes become progressively constrained, ultimately preventing radicle emergence below the base temperature (Simon, 1976; Bradford, 2002).

- **Base Temperature and Thermal Time Concept**

The concept of base temperature (Tb) refers to the theoretical minimum temperature below which germination does not occur. Germination rate decreases linearly as temperature

declines toward Tb, reflecting the temperature dependence of biochemical reactions and enzymatic kinetics (Bradford, 2002). This relationship forms the basis of the thermal time model, which explains germination timing as a function of accumulated temperature units above Tb (Bradford, 2002).

The low-temperature limit for germination is determined by fundamental physiological constraints rather than simple environmental limitation. When temperature falls below Tb, embryo growth potential becomes insufficient to overcome mechanical resistance of surrounding tissues (Simon, 1976).

- **Membrane Structure and Phase Transitions**

Low temperature directly influences cellular membranes, which play a central role during imbibition and metabolic reactivation. During the early stages of germination, membranes transition from a dry, gel-like state to a hydrated, functional configuration. Reduced temperatures decrease membrane fluidity due to lipid phase transitions, impairing permeability and solute transport (Bewley & Black, 1994; Vertucci & Leopold, 1987).

Membrane rigidification under cold conditions may lead to:

- Altered ion flux
- Reduced membrane-bound enzyme efficiency
- Disrupted compartmentalization

These structural changes delay progression from water uptake (Phase I) to metabolic activation (Phase II) of germination (Bewley & Black, 1994).

- **Enzymatic and Metabolic Constraints**

Germination requires coordinated activation of hydrolytic enzymes responsible for mobilizing stored reserves. Because enzyme activity is temperature-dependent, low temperatures significantly reduce catalytic efficiency and metabolic flux (Bewley & Black, 1994).

Cold stress slows:

- Amylase-mediated starch hydrolysis
- Protease activity
- Lipid mobilization
- Respiratory metabolism

Reduced mitochondrial respiration limits ATP production, thereby restricting embryo growth potential necessary for radicle protrusion (Bewley & Black, 1994; Bradford, 2002).

- **Hormonal Regulation Under Low Temperature**

Seed germination is regulated by the antagonistic interaction between abscisic acid (ABA) and gibberellins (GA). Low temperature conditions can modify hormonal balance by maintaining elevated ABA levels or reducing GA synthesis and signaling, thereby reinforcing dormancy-like states (Finch-Savage & Leubner-Metzger, 2006).

This hormonal modulation delays the transition from quiescence to active growth and integrates environmental temperature signals with endogenous developmental regulation (Finch-Savage & Leubner-Metzger, 2006).

- **Oxidative Metabolism and Redox Regulation**

Reactive oxygen species (ROS) function as signaling molecules during germination, contributing to cell wall loosening and reserve mobilization. However, low temperature may disrupt redox homeostasis, resulting in oxidative imbalance that impairs cellular structures and metabolic pathways (Bailly, 2004).

Efficient antioxidant systems are therefore essential for maintaining cellular integrity under suboptimal thermal conditions (Bailly, 2004).

- **Ecophysiological Implications**

Species-specific variation in base temperature reflects ecological adaptation. Seeds from temperate regions generally possess lower Tb values, allowing germination at relatively low temperatures, whereas tropical species exhibit higher minimum thresholds (Simon, 1976; Bradford, 2002).

d) Water

Water is utilized by the embryo to induce cellular swelling, which leads to radicle emergence (Domonique, 2007).

Water absorption initiates tissue hydration, activates metabolic pathways, and enables cell expansion. The swelling of cells is a prerequisite for the protrusion of the radicle, marking the visible onset of germination.

I.4 Salinity Stress:

I.4.1 Concept of Salinity Stress

Salinity stress refers to the condition in which excessive soluble salts accumulate in the soil solution to levels that impair plant growth and productivity. It is one of the most serious abiotic constraints in agriculture, particularly in arid and semi-arid regions. Globally, 4–7% of arable land is affected by salinity (Ebert, 2000), and nearly 380 million hectares of potentially cultivable land are restricted by salt accumulation (Lambers, 2003).

Salinity commonly develops due to high evaporation rates, insufficient rainfall for leaching, poor drainage, and the use of mineralized irrigation water (Ben Asher *et al*., 2002).

I.4.2 Nutritional Imbalance Under Salinity

Salinity alters nutrient availability in the soil and nutrient uptake by plants. Elevated salt concentrations can:

- Reduce nutrient absorption
- Disturb ion balance within tissues
- Create antagonistic interactions among mineral elements

Salinity-induced imbalances commonly affect essential macronutrients such as potassium, calcium, magnesium, and phosphorus (Grattan and Grieve, 1999). These nutrients are critical for:

- Enzyme activation
- Membrane stability
- Osmoregulation
- Energy metabolism

Micronutrient availability may also fluctuate under saline conditions, though responses vary among species and environmental contexts (Grattan and Grieve, 1999).

I.5. Role of Sodium Chloride (NaCl) in Seed Germination

Sodium chloride (NaCl) is the predominant salt responsible for salinity stress in agricultural systems, particularly in arid and semi-arid environments. At the level of seed germination, its effects are primarily mediated through two interconnected mechanisms: osmotic constraint

and ionic toxicity, both of which interfere with the physiological and biochemical processes governing embryo reactivation (Ebert, 2000; Marschner, 1995).

Unlike later developmental stages, germination represents a highly sensitive phase in which metabolic activation, reserve mobilization, and embryonic growth depend strictly on water availability and ionic balance. Consequently, NaCl acts as a critical limiting factor during the transition from seed dormancy to active growth.

I.5.1 Osmotic Effects of NaCl During Germination

a) Reduction of Water Uptake (Imbibition Constraint)

The initiation of germination depends on imbibition, a process driven by the gradient between seed water potential and the surrounding medium. The presence of NaCl in the germination environment lowers the external water potential, thereby reducing this gradient and limiting water influx into the seed (Ebert, 2000).

Even when water is physically present, it becomes physiologically unavailable, delaying or preventing the rehydration of cellular structures. This directly affects:

- Reconstitution of membrane integrity
- Activation of enzymatic systems
- Initiation of metabolic pathways

Since imbibition represents the first phase of germination, osmotic stress acts as an early inhibitory factor, often preceding any visible symptoms.

b) Effects on Metabolic Activation (Lag Phase)

Following initial water uptake, seeds enter a metabolically active phase characterized by enzyme synthesis and reserve mobilization. Under saline conditions, reduced hydration levels limit:

- Enzyme activation (e.g., amylases, proteases)
- Substrate diffusion within tissues
- Respiratory metabolism

This results in a slowdown of biochemical processes necessary for embryo growth (Bewley & Black, 1994). The seed may remain in a prolonged lag phase without progressing toward radicle emergence.

c) Inhibition of Embryo Growth and Radicle Emergence

Germination is completed when the embryonic axis generates sufficient growth force to rupture the seed coat. Osmotic stress reduces cell turgor pressure, which is essential for:

- Cell elongation
- Tissue expansion
- Mechanical breakthrough of surrounding structures

As a result, even metabolically active seeds may fail to achieve radicle protrusion. This explains why salinity often reduces germination percentage and germination speed simultaneously (Ebert, 2000; Bewley & Black, 1994; Bradford, 2002).

I.5.2 Ionic Toxicity at the Seed Level

a) Accumulation of Na^+ and Cl^- in Embryonic Tissues

As exposure to NaCl continues, sodium (Na^+) and chloride (Cl^-) ions progressively penetrate seed tissues. When their concentration exceeds the buffering and compartmentalization capacity of the cells, toxic effects emerge (Marschner, 1995).

At the cellular level, excessive ions may:

- Disrupt enzymatic activity
- Alter protein structure
- Interfere with mitochondrial respiration
- Damage membrane systems

b) Membrane Destabilization and Cellular Dysfunction

During germination, membranes transition from a dry, disorganized state to a functional, semi-permeable system. High Na^+ concentrations can destabilize membrane structure by displacing essential cations such as Ca^{2+} (Ebert, 2000).

This leads to:

- Increased membrane permeability
- Loss of solute compartmentalization
- Disrupted ion gradients

As a consequence, cellular homeostasis is compromised, reducing the seed's capacity to sustain metabolic activation.

I.5.3 Nutritional Imbalance in Germinating Seeds

a)Ionic Competition and Selectivity

NaClinduced salinity disrupts the internal balance of essential nutrients through ionic competition. Sodium competes with key cations such as potassium (K^+) and calcium (Ca^{2+}) for uptake and binding sites (Grattan and Grieve, 1999).

This results in altered ionic ratios, particularly:

- Na^+/K^+
- Na^+/Ca^{2+}

These ratios are more critical than absolute ion concentrations because they determine enzymatic efficiency and cellular stability.

b) Potassium (K^+) Disruption

Potassium plays a central role in:

- Enzyme activation
- Osmotic regulation
- Maintenance of cellular turgor

Under saline conditions, Na^+ competes with K^+, leading to reduced K^+ availability within embryonic tissues. This imbalance inhibits metabolic reactions essential for reserve mobilization and growth (Hu and Schmidhalter, 2005).

c) Calcium (Ca^{2}+) Deficiency and Membrane Integrity

Calcium is essential for membrane stabilization and signaling processes. Elevated Na+ levels can displace Ca^{2}+ from membrane binding sites, resulting in:

- Reduced membrane stability
- Impaired signal transduction
- Increased susceptibility to leakage and damage

This contributes significantly to the inhibition of germination under saline conditions (Grattan and Grieve, 1999).

I .5.4 Integration of Osmotic and Ionic Effects

Salinity stress during germination develops in two overlapping phases:

a) Initial Osmotic Phase

- Immediate reduction in water uptake
- Delayed imbibition
- Inhibition of metabolic activation

b)Secondary Ionic Phase

- Progressive accumulation of Na^+ and Cl^-
- Enzymatic inhibition
- Membrane destabilization
- Nutritional imbalance

The transition between these phases depends on both salt concentration and duration of exposure. While osmotic effects dominate early germination, ionic toxicity becomes increasingly significant over time (Ebert, 2000; Marschner, 1995)

I.6. Plant Tolerance Mechanisms to Salinity

Higher plants exhibit different physiological behaviors under saline conditions and can be broadly categorized as includers and excluders (Marschner, 1995). Includer plants, mostly halophytes, accumulate significant amounts of salts to maintain water uptake. In contrast, excluder plants—mainly non-halophytes, including fruit trees—depend on their ability to restrict ion translocation to shoots or to compartmentalize toxic ions within cellular structures (Marschner, 1995). The tolerance of horticultural crops to salinity is influenced by environmental conditions and plant factors such as growth stage and genetic background (Kozlowski, 1997).

Plant tolerance mechanisms to salinity involve several integrated physiological and nutritional processes.

I.6.1 Regulation of Sodium (Na+) and Chloride (Cl-)

Under saline conditions, Na+ and Cl- concentrations in plant tissues often exceed those of essential nutrients. Excessive accumulation of these ions can disrupt cellular metabolism and induce toxicity. A key tolerance mechanism in fruit tree species involves limiting the transport of saline ions to sensitive tissues or compartmentalizing them within vacuoles to reduce cytoplasmic toxicity (Marschner, 1995).

Tolerance may therefore depend on:

- The plant's ability to regulate Na^+ transport.
- Compartmentalization of Na^+ and Cl^- within cells.
- Physiological capacity to function despite elevated internal ion concentrations.

In some tolerant cultivars of olive, reduced Na^+ transport to leaves has been associated with increased salt tolerance (Tabatabaei, 2006). However, tolerance can also involve the ability to withstand relatively high concentrations of Na^+ and Cl^- in leaf tissues without severe disruption of metabolic processes.

I.6.2 Maintenance of Nutritional Balance

Salinity frequently disturbs mineral nutrition due to ionic competition and altered soil solution chemistry (Grattan and Grieve, 1999). Elevated Na^+ and Cl^- concentrations may reduce the availability and uptake of Ca^{2+}, K+, Mg^{2+} and $NO3^-$, leading to nutritional imbalances.

a)Potassium (K+)

Potassium plays a central role in osmotic adjustment, enzyme activation, and stomatal regulation. Under salt stress, Na^+ may compete with K^+ for uptake and transport, leading to Na^+/K+ antagonism (Tejera *et al.,* 2006).

A major tolerance mechanism involves:

- Selectivity for K^+ over Na^+.
- Preferential loading of K^+ into the xylem.
- Maintenance of high K^+ concentrations in leaves.

Many crop species exhibit selective uptake of K^+ over Na+ as a strategy to resist salt stress (Hu & Schmidhalter, 2005).

b)Calcium (Ca^{2+})

Calcium is critical for membrane stability and regulation of ion transport. Salinity, particularly Na^+ salts, may reduce Ca^{2+} availability by displacing Ca^{2+} from extracellular binding sites (Grattan and Grieve, 1999). Reduced Ca^{2+} transport to growing tissues is a common consequence of salt stress (Ebert, 2000).

Salt tolerance may thus be associated with:

- The ability to retain Ca^{2+} in leaf tissues.
- Maintenance of balanced Na^+/Ca^{2+} ratios.

- Prevention of membrane destabilization under ionic stress (Hu and Schmidhalter, 2005).

c) Nitrogen (N), Phosphorus (P), Magnesium (Mg^{2+}), and Micronutrients

The effect of salinity on other nutrients is variable (Grattan and Grieve, 1999). Some studies report reduced P concentrations under saline conditions, whereas others show no significant change. Micronutrient responses to salinity are inconsistent and species-dependent. Thus, a stable nutrient status under saline conditions contributes to tolerance by sustaining essential physiological functions.

I.6.3 Osmotic Adjustment

Salinity reduces soil water potential, creating osmotic stress that limits water uptake (Ebert, 2000). To counteract this, plants perform osmotic adjustment by enhancing inorganic ion uptake and accumulation, allowing continued water absorption and turgor maintenance.

Fruit trees may respond by:

- Increasing uptake of compatible inorganic ions.
- Adjusting internal osmotic potential.
- Maintaining cellular hydration despite saline environments (Ebert, 2000).

Osmotic adjustment is therefore a central physiological mechanism enabling plants to survive under moderate salinity.

I.6.4 Growth Modulation and Biomass Allocation

Salinity affects vegetative growth by suppressing shoot development, leaf initiation, leaf expansion, and internode elongation (Noble and West, 1989; Kozlowski, 1997). Reduced vegetative growth under salinity can be interpreted as an adaptive response that limits metabolic demand and ion accumulation.

Adaptive growth responses may include:

- Reduction in leaf production.
- Leaf shedding.
- Altered biomass allocation between roots and shoots.

Chapter II

General Overview of the

(Ceratonia siliqua L.)

Chapter II: General Overview of *(Ceratonia siliqua L)*

II.1.General description of the *Ceratonia siliqua L*

Figure 1: image The *(Ceratonia siliqua L)*

Site web https://www.google.com/search

The *Ceratonia siliqua L* tree is a perennial, evergreen fruit tree belonging to the family *Fabaceae (Leguminosae).* It is native to the Mediterranean basin, which is considered its main center of origin and diversification. Over time, the species has spread widely and is now cultivated or naturalized in many regions of the world with Mediterranean or semi-arid climates, including Southern Europe, North and East Africa, the Middle East, North and South America, Australia, and South *Africa* (Batlle & Tous, 1997; Viruel *et al*., 2020).

The *Ceratonia siliqua L* tree is characterized by slow growth, exceptional longevity that may exceed several hundred years, and a high tolerance to harsh environmental conditions. Mature trees generally reach heights ranging from 10 to 15 m and develop a broad, dense canopy composed of dark green, leathery, evergreen leaves. These characteristics make *the 1* tree particularly valuable for soil conservation and erosion control in degraded landscapes (Mahdad, 2013; Plants, 2023).

One of the most remarkable features of *C. siliqua.L* is its high resistance to drought. This tolerance is mainly attributed to a deep and well-developed root system that enables the tree to

access water from deep soil layers. The *Ceratonia siliqua.L* tree can thrive on poor, rocky, calcareous, and low-fertility soils, making it suitable for reforestation programs, the rehabilitation of marginal lands, and combating desertification in arid and semi-arid regions (Batlle & Tous, 1997).

The fruit of the *Ceratonia siliqua L* is a fleshy pod rich in sugars, dietary fibers, and essential nutrients. Both the pulp and seeds possess significant economic and nutritional value. They are widely used in human and animal nutrition, as well as in the food, pharmaceutical, and cosmetic industries. *Ceratonia siliqua L* powder, obtained from the pod pulp, is commonly used as a natural cocoa substitute due to its sweet taste and absence of caffeine *(*Rtibi *et al*., 2015; Plants, 2023).

In traditional medicine, various parts of the *Ceratonia siliqua L* including leaves, bark, seeds, and pods—have been used to treat several ailments such as diarrhea, diabetes, and hypertension. Recent scientific studies have confirmed multiple pharmacological properties of *Ceratonia siliqua .L*, including antioxidant, antidiarrheal, antibacterial, anti-inflammatory, and anti-ulcer activities, highlighting its potential as a functional food and natural therapeutic agent *(*Rtibi *et al*., 2015; Plants, 2023).

In North African countries, particularly Morocco and Algeria, the *Ceratonia siliqua L* plays an important economic, ecological, and social role. It contributes to sustainable rural development by enhancing the value of marginal lands unsuitable for other crops and provides income opportunities for local populations, despite a decline in cultivated areas in certain regions over recent decades (Batlle & Tous, 1997; FAO, 2022).

II.2.Classification of *Ceratonia siliqua L*

Ceratonia siliqua L., commonly known as the *Ceratonia siliqua L*, belongs to the *Fabaceae* family and is classified among flowering plants *(angiosperms),* dicotyledons, within the order *Fabales.*

Its systematic botanical classification helps clarify its evolutionary position within the legume family (Lewis *et al*., 2005; Ait Chitt *et al*., 2007).

Table.1: Botanical Classification of *Ceratonia siliqua L* (Sallouh et Nouioui, 2019).

Rank	**Classification**
Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Division	*Magnoliophyta*
Subdivision	*Angiosperms*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Caesalpinioideae*
Genus	*Ceratonia*
Species	*Ceratonia siliqua L.*
Common Name	*Carob tree*

II.3.Botanical description

II.3.1 The Tree

The tree *Ceratonia siliqua L.* is a typically Mediterranean species, characterized by a chromosome number of **2n = 24**. It develops mainly in warm climates and can reach a height of **7–10 meters**, or even **15–20 meters under optimal conditions**, with a trunk circumference at the base ranging from **2 to 3 meters** (Benmahioul *et al.*, 2021; Turnbull *et al.*, 2006). Its crown is notably wide-spreading and rounded in shape. Due to its slow growth rate, the *Ceratonia siliqua* tree can live for more than **200 years** (Figure 02) (Rejeb *et al*., 1991; Benmahioul *et al*., 2011).

The *Ceratonia siliqua L* tree is a **xerophilous species**, meaning it is well adapted to arid environments. Because of its ecological characteristics, it is commonly used in **reforestation programs** aimed at combating soil erosion and desertification. Furthermore, it is highly valued as an **ornamental plant** and is frequently incorporated into landscaping projects (Figure 02) (Boudy, 1950; Rejeb *et al*., 1991; Biner *et al*., 2007).

Figure.2: tree *Ceratonia siliqua L* (Original photo, 2021)

II.3.2 Root System

The *Ceratonia siliqua L* tree develops a **taproot system** that can reach up to 18 meters in depth. The root structure and the composition of root exudates change during the plant's development and are modulated by **environmental conditions**, such as water availability and temperature (Aafi, 1996; Gharnit, 2003).

Figure.3: Root system of the *Ceratonia siliqua L* tree *(*Mahdad, 2013*).*

II.3.3 Stem

The trunk of the *Ceratonia siliqua L* tree is distinguished by its **robustness and thickness**, with well-visible sap channels, particularly near the thickest roots, giving it **sinuous curves**, especially in certain varieties In large, mature trees, the trunk adopts **twisted and winding forms**, with an average diameter of approximately **50 cm**, varying according to the age of the tree (Albanell, 1990). The trunk circumference at the base generally ranges between **2 and 3 meters** (Melgarejo & Salazar, 2003).

Figure.4 : The trunk of the *Ceratonia siliqua L* (site google)

II.3.4 Leaves

the leaves of the *Ceratonia siliqua L* tree are **evergreen, alternate, and paripinnate**, meaning they consist of an even number of leaflets. They typically measure **10–20 cm in length** and are composed of **8–15 opposite leaflets** arranged along the rachis. Individual leaflets are **oval to elliptic**, leathery, entire, and slightly notched at the apex, with lengths ranging from **3 to 7 cm** (Ait Chitt *et al.*, 2007; Sbay, 2008).

The upper surface of the leaflets is dark glossy green, while the lower surface is lighter and matte in appearance (Figure 5). The petiole is short and characterized by grooves on its inner surface, and the rachis is thick and firm.

As an evergreen species, the *Ceratonia siliqua L* tree retains its foliage throughout the year and does not shed its leaves in autumn. However, it undergoes a **partial leaf fall approximately every two years**, usually around the month of July. These leaves are subsequently renewed in spring (March–April). This phenological behavior contributes to the ecological adaptability of the species in Mediterranean environments (Baumel *et al*., 2018).

Figure.5: *Ceratonia siliqua L* tree leaf

II.3.5 Flowers

The *Ceratonia siliqua L* tree is mostly **dioecious**, sometimes hermaphroditic, and rarely monoecious.

- **Male trees:** long stamens, undeveloped pistil; used as pollinators.
- **Female trees:** developed pistil, rudimentary stamens; most abundant.
- **Hermaphroditic trees:** well-developed stamens and pistils

Flowering occurs from **August to October**, sometimes from **September to November**, depending on local climatic conditions. Male trees are sterile and unproductive. **Fruit set** occurs from **July to December** of the year following flowering, depending on the region and cultivar (Benmahioul *et al.,* 2011;Chitt *et al*., 2007.)

Figure.6: *Ceratonia siliqua L* flowers in clusters

(https://www.bio-enligne.com/produits/136-caroubier.html)

II.3.6 Fruits

Fruits are **elongated pods**, green at first and turning brown at maturity, measuring **10–30 cm in length and 2–3.5 cm in width**. Each pod contains **12–16 brown seeds**, separated by pulp partitions. The pulp is **sweet**, rich in **carbohydrates, fibers, proteins, minerals, and polyphenols** (Batlle & Tous, 1997;Tous *et al*., 2013.).

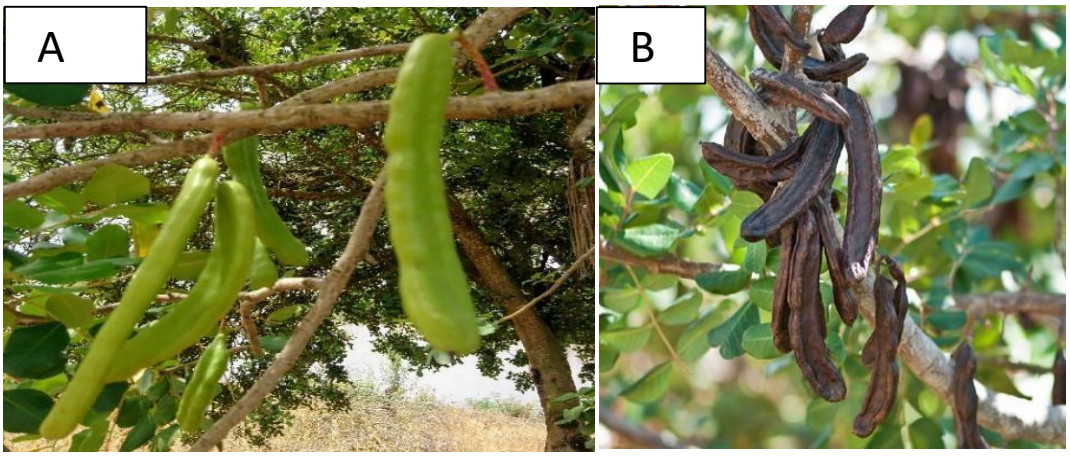

Figure.7 *C siliqua L* fruit — (A) Young *C siliqua L* pod (original photo, 2021), (B) Mature carob pod

(Source B : https://www.gastronomiac.com/glossaire_des_produi/caroube/)

II.3.7 Seeds

Seeds account for **10% of the total fruit weight**. They are used for **extracting galactomannans and** *Ceratonia siliqua L* **gum**, which are important in **food, pharmaceutical, and cosmetic industries**. Carob seed flour contains **52% protein**, rich in lysine and arginine, and 27% carbohydrates (Ayaz, 2009)

Figure.8: *Ceratonia siliqua L* seeds (Original photo, 2026)

II.3.8 Wood

The wood of *the Ceratonia siliqua L* is pale yellow when young, gradually turning pink-veined and eventually dark red and hard with age. It is highly valued in woodworking, marquetry, gunsmithing, wheelwrighting, and also for charcoal production (*BenMahioul et al*., 2011).

Figure.9 : The wood of the *Ceratonia siliqua L.* (Photo originale 2026)

II.3.9 Branches

The characteristics of *Ceratonia siliqua L* branches vary according to their age (*Albanell, 1990):*

- **Primary branches** of older trees are generally thick, tortuous, and tend to grow horizontally due to their weight and pruning practices. Their main role is to support other branches, although they can occasionally bear fruit.
- **Secondary branches** are of medium size and tend to grow more or less upright, particularly in the upper part of the canopy. These branches constitute the main productive structure of the tree.
- **Young branches or twigs**, smaller in size, are located in the outer part of the canopy or in the active growth zone. They are flexible, with smooth bark covered by lenticels that facilitate gas exchange with the atmosphere. Depending on the variety, their coloration in the budding area is typically yellowish-green or reddish.

Figure II.10: The Branches of the *Ceratonia siliqua L*

II.4.Chemical Composition of *Ceratonia siliqua L*

The chemical composition of the various components of *Ceratonia siliqua L* depends on the cultivar, the cultivation region, and the harvest date. In Turkey, a study comparing the main sugar profiles of pulp from cultivated and wild *Ceratonia siliqua L* varieties showed that sucrose is the predominant sugar, with smaller amounts of glucose and fructose in wild varieties compared to cultivated ones. However, the proportion of individual sugars relative to total sugars was similar in both cases (Biner *et al.*, 2007).

The fleshy pulp consists of approximately 50% sugars (mainly sucrose, glucose, fructose, and maltose), 18% cellulose and hemicellulose, 16–20% tannins, and 1–2% proteins. Unlike cocoa, *Ceratonia siliqua L* contains neither theobromine nor caffeine. It is, however, rich in calcium, phosphorus, potassium, magnesium, and pectin (Aafi, 1996).

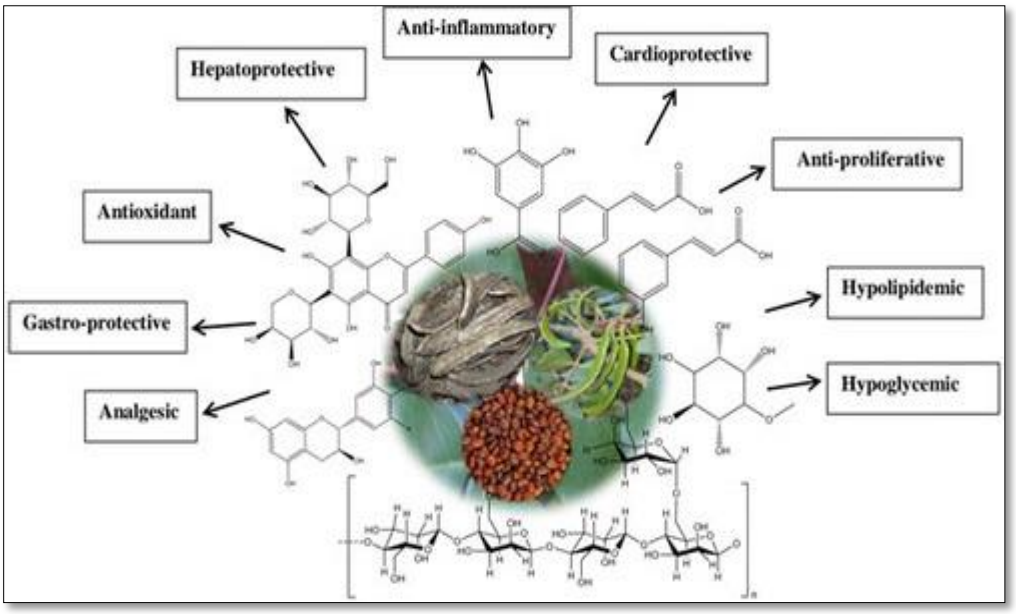

Figure.11 The chemical composition of the various components of *Ceratonia siliqua L*

II.4.1 Mineral composition

The mineral composition (mg/100 g dry weight) is as follows: K = 970; Ca = 300; P = 71; Mg = 60; Fe = 1.88; Mn = 1.29; Cu = 0.85; Zn = 0.75 (Ayaz et al., 2007). It should be noted that the pulp is particularly rich in potassium and calcium, which is highly beneficial for human and animal nutrition (more than double the calcium content of whole milk, which is 119 mg/100 g).

Table.2: Chemical composition of *Ceratonia siliqua (Kicher & Ladjouzi, 2016)*

Pulp (90%)	**Seed (10%)**
Carbohydrates: 48–72%	Seed coat (cuticle): 30–33%
Proteins: 1–2%	
Fats: 0.5–0.7%	
Cellulose and hemicellulose: 18%	Endosperm (albumen): 42–46%
Minerals (Ca, Mg, K, P)	
Pectins and fibers: 4.2–9.6%	Embryo (germ): 23–25%
Ash: 1.5–2.4%	
Polyphenols: 16–20%	

II.5.Uses of the *Ceratonia siliqua L*

The *Ceratonia siliqua L* is recognized as a multipurpose Mediterranean species with considerable ecological, socio-economic, nutritional, and industrial value. Its resilience to drought and poor soils, together with the wide range of uses of its different organs, makes it an important resource for sustainable development in arid and semi-arid regions (Giovanni Battista Batlle & Joan Tous, 2007; Konstantinos Tziouvaras *et al.*, 2012). Nearly all parts of the tree — leaves, flowers, pods, seeds, wood, bark, and roots — are exploitable in agriculture, food processing, medicine, and environmental management.

II.5.1.1 The Tree

The *Ceratonia siliqua. L* tree develops a large, dense, rounded canopy that provides deep shade and contributes to microclimate regulation. Its structure and evergreen foliage help reduce fire spread and soil desiccation, making it useful in wildfire risk mitigation and landscape protection (Juli G. Pausas & Jon E. Keeley, 2014).

It is frequently planted as a windbreak and plays a protective role against desertification and environmental degradation (Mohamed Rejeb *et al*., 2003). Reforestation using *Ceratonia siliqua L* trees is an effective approach to stabilizing soils and rehabilitating degraded lands affected by erosion and overgrazing (Antonio Sánchez-Gómez *et al*., 2016).

Thanks to its tolerance to drought, air pollution, salinity, and nutrient-poor soils, *the Ceratonia siliqua L* tree is also planted as an ornamental and roadside tree in urban and peri-urban environments (Stefano Mancuso, 2015).

II.5.1.2 Fruit

The *Ceratonia siliqua L* fruit is a pod containing hard seeds embedded in a sweet, fibrous pulp rich in sugars and bioactive compounds (Batlle & Tous, 2007).

Ceratonia siliqua. L pods are widely used as livestock feed due to their high carbohydrate content and nutritive value comparable to cereal grains (Nissim Silanikove *et al.*, 2006). They provide high energy content and improve palatability in animal diets.

Ceratonia siliqua L pulp has been traditionally used to support cardiovascular health by helping reduce serum cholesterol and blood pressure levels (Hans-Joachim Zunft *et al*., 2003). Recent

studies confirm its hypocholesterolemic, antioxidant, antidiarrheal, and digestive regulatory *effects* (Kais Rtibi *et al.*, 2017; Soumaya Benchikh *et al*., 2021).

Traditionally, *Ceratonia siliqua L* pulp has been used to treat diarrhea, gastritis, respiratory infections, and digestive disorders due to its tannin and polyphenol content (Rtibi *et al.*, 2017).

Ceratonia siliqua L is naturally low in sodium and rich in potassium, making it suitable for diets aimed at managing hypertension and cardiovascular diseases (Zunft *et al*., 2003).

Two principal products are derived from *Ceratonia siliqua L* pods:

- *Ceratonia siliqua L* flour, obtained by drying, roasting, and grinding the pulp (Dimitrios Makris *et al.*, 2011).
- This flour is widely used in the food industry and has also been investigated as a renewable substrate for bioethanol production (Oscar Sánchez *et al*., 2010).

Ceratonia siliqua L powder is frequently used as a cocoa substitute because it is caffeine-free and theobromine-free, naturally sweet, and lower in fat, making it suitable for dietetic and functional foods (Arianna Papagiannopoulos *et al*., 2004; Maria Avallone *et al*., 2018).

II.5.1.3 Leaves and Flowers

Ceratonia siliqua L leaves are rich in tannins and phenolic compounds. Their digestibility in ruminant diets can be improved by treatments such as polyethylene glycol (PEG), enhancing nutritional *value* (Silanikove *et al.*, 2006). Leaf extracts have demonstrated antioxidant and antiproliferative activities in laboratory studies (Rtibi *et al*., 2017; Benchikh *et al.*, 2021).

Leaves are also used as supplementary fodder during feed shortages in Mediterranean pastoral systems.

Ceratonia siliqua L flowers provide an important nectar source for bees, making the species valuable for apiculture. The tree is considered melliferous and contributes to the production of high-quality honey (José Antonio González *et al*., 2011).

II.5.1.4 Bark and Wood

Ceratonia siliqua L bark contains tannins that have been used in leather processing and natural dye production (Antonio Pizzi, 2008).

The wood is dense, hard, and durable, making it suitable for carpentry, charcoal production, fuelwood, and the manufacture of small tools and household utensils (Batlle & Tous, 2007; Pizzi, 2008).

Figure.12: Pulp flour (A); seed gum (B); and confectionery products made from *Ceratonia siliqua L* (chocolate, biscuits, jam, etc.) (Mahdad, 2013)

II.5.2 Uses and Benefits of the *Ceratonia siliqua L*

According to Boublenza *et al*. (2019), the *Ceratonia siliqua L* holds significant economic and socio-economic importance. Today, it is considered one of the most productive fruit and forest trees. All parts of the tree — leaves, flowers, pulp, seeds, and bark — are valuable, particularly the fruit, which is used in various fields such as food, pharmaceuticals, cosmetics, and tanning.

II.5.2.1. Use in the Food Industry

In the food industry, two *Ceratonia siliqua L* -derived products are widely used: *C siliqua L* (flour and gum).

Ceratonia siliqua L pods have long been used as raw materials for food additives (Biner *et al*., 2007). *C siliqua L* gum, also known by its code E-410, is utilized as a thickener, stabilizer, binder, gelling agent, or dispersant in food products. *C siliqua L* gums are also used as raw materials in printing, photography, textiles, pharmaceuticals, and cosmetics (Batlle *et al.,* 1997).

Due to its sweetness, chocolate-like flavor, and low cost, ground *Ceratonia siliqua L* pods are commonly used as a cocoa substitute in confectionery, biscuits, processed products, and beverages in the Mediterranean region (Ayaz *et al.,* 2009). Furthermore, C siliqua L powder derived from the pods serves as a natural sweetener, making it an alternative to cocoa. A key advantage of its use is that it does not contain stimulants such as caffeine or theobromine, unlike chocolate (Bengoechea *et al.,* 2008).

II.6.Biogeographical Distribution

II.6.1 Global Distribution

The tree *Ceratonia siliqua L* is a typically Mediterranean species, originating from the Middle East, and distributed throughout the Mediterranean basin, including Asia Minor, North Africa, Southern Europe, and the Iberian Peninsula (Fig. 13). It is currently found from Spain and Portugal to Turkey, Syria, Yugoslavia, Morocco, Algeria, Tunisia, Libya, Egypt, Greece, Lebanon, Cyprus, Italy, and France (Boudy, 1950; Rejeb, 1995; Sbay & Abourouh, 2006; Abi Azar, 2007).

Ceratonia siliqua L has also been successfully introduced to other countries with Mediterranean climates, such as Australia, South Africa, the United States (Arizona, Southern California), the Philippines, and Iran (Evreinoff, 1947; Batlle & Tous, 1997). The distribution of tree species like *C. siliqua* is generally limited by cold stress (Mitrakos, 1981). In contrast, *Ceratonia oreothauma*, which appears more sensitive to cold, has a very restricted distribution, confined to Oman and Somalia (Hillcoat *et al*., 1980).

Historically, *Ceratonia siliqua L* was propagated by the Greeks, followed by Arabs and Berbers of North Africa, spreading to Greece, Italy, Spain, and *Portugal* (Rejeb, 1995; Gharnit, 2003), and later introduced to the Americas and Australia by the Spanish. Today, it is also found in the Philippines, Iran, South Africa, and India (Berrougui, 2007).

In low Mediterranean zones (0–500 m, occasionally up to 900 m), *C. siliqua L* is a dominant species characteristic of sclerophyllous maquis (Folch & Guillen, 1981), adapting to various soil types.

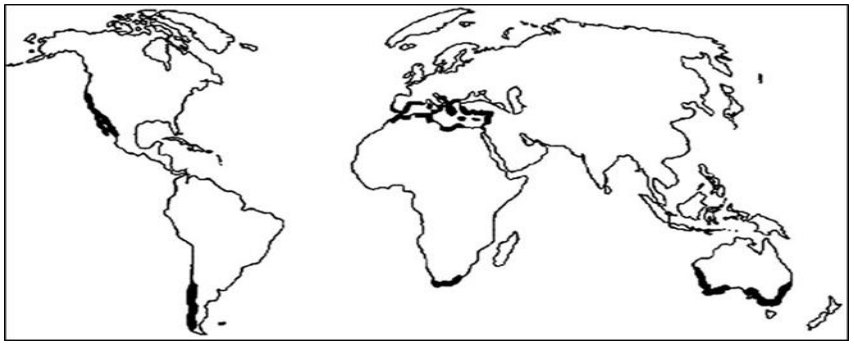

Figure 13: World map of *Ceratonia siliqua L* distribution in Algeria

II.6.2. Mediterranean Region

Melgarejo and Salazar (2003) consider the Mediterranean basin the center of *Ceratonia siliqua L* diversity. Even if it is not the original center of the species, it is the area with the greatest genetic diversity, making it the most promising region for finding new genetic material. In Europe and North Africa, *C. siliqua L* occurs naturally in association with olive (*Olea europaea*), thuya, and pine trees (Aafi, 1996; Batlle & Tous, 1997).

In Morocco, *Ceratonia siliqua L* is found in plains and mid-altitude mountains of the Rif, Middle Atlas, High Atlas, and Anti-Atlas, in humid, subhumid, semi-arid, and coastal arid bioclimates with warm to temperate variations. It is often associated with olive, mastic, thuya, or argan trees. The main wild population occurs between 600–1000 m, protected from wind and frost (Ait Chitt *et al.*, 2007; Konate, 2007).

In Tunisia, *Ceratonia siliqua L* grows naturally in humid, subhumid, and upper semi-arid zones, often in association with olive and mastic. However, agricultural expansion has reduced these wild populations (Rejeb, 1989; 1995).

II.6.3 Algeria

In Algeria, *C. siliqua L* is frequently cultivated in the Saharan Atlas and is common in the Tell region (Quezel & Santa, 1962). It grows naturally with almond, olive, and *Pistacia atlantica* in semi-arid, subhumid, and humid zones, at altitudes of 100–1300 m in cool valleys that protect it

from frost, with temperatures ranging from 5°C to 20°C and annual rainfall between 80–600 mm (Batlle & Tous, 1997; Benmahioul *et al.,* 2011).

Preferred locations include sunny hills in coastal and subcoastal areas, such as the Algerian Sahel, Dahra, Kabylie, and the valleys of Sommam and Oued Isser. It extends to Bou–Saâda but does not bear fruit there, and to the Traras region north of Tlemcen (Lavallée, 1962; Zitouni, 2010). According to *Gaouar (2011),* the distribution in Tlemcen includes Sidi M'djahed, Sebra, Henaya, Tlemcen, Aïn Tellout, Sidi Abdli, Remchi, Ben Sekran, Aïn Youcef, and Beni Saf.

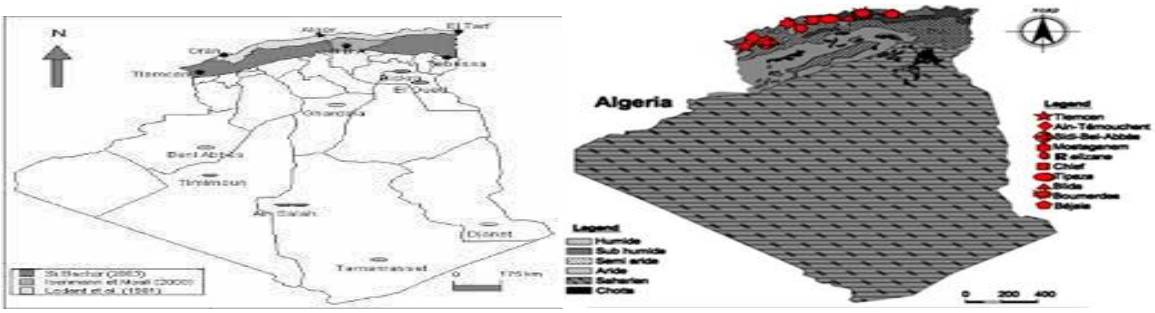

Figure.14: Distribution of the *Ceratonia siliqua L* tree in Algeria according to bioclimatic zones (A.N.R.H, 2004; Boublenza *et al*., 2019).

II.7.Economic and Ecological Importance of the *Ceratonia siliqua L*

The tree *Ceratonia siliqua L* is a key Mediterranean species with both ecological and economic value. Ecologically, it stabilizes soils, reduces erosion, and combats desertification due to its deep roots, drought tolerance, and adaptability to poor *soils* (Mohamed Rejeb *et al*., 2003; Antonio Sánchez-Gómez *et al.,* 2016). It enhances biodiversity by providing habitat and food for wildlife and supports ecosystem rehabilitation in degraded or marginal lands.

Economically, *Ceratonia siliqua L* pods serve as livestock feed and are processed into flour, powder, and locust bean gum for the food, pharmaceutical, and cosmetic industries (Dimitrios Makris *et al*., 2011; Oscar Sánchez et al., 2010). C. siliqua L products are increasingly valued for their antioxidant properties and potential health benefits, while the tree also supports apiculture and sustainable agroforestry systems (Kais Rtibi *et al*., 2017; Soumaya Benchikh *et al.,* 2021).

Chapter III

Materials and Methods

III.1. Experimental Site

the experimental work was carried out in the Plant Biology Laboratory, Faculty of Natural and Life Sciences, at the Université Dr Moulay Tahar Saïda, Algeria.

Germination assays were conducted under controlled laboratory conditions. Petri dishes were incubated in a growth chamber at a constant temperature ranging from 27 to 28 °C, which is considered optimal for the germination of Mediterranean woody species. Temperature is a key environmental factor influencing enzymatic activity and metabolic processes involved in seed germination (Bewley *et al.*, 2013).

In addition, a cold treatment at 5 °C was applied to evaluate the effect of low temperature on germination behavior. The experimental period lasted 15 days, in accordance with standard germination testing procedures for tree species (ISTA, 2020).

III.2. Plant Material

The plant material consisted of mature seeds of *Ceratonia siliqua L.*, a species known for its hard and impermeable seed coat, which induces physical dormancy and requires pre-treatment to enhance germination (Baskin & Baskin, 2014).

Healthy, uniform, and undamaged seeds were selected in order to reduce experimental variability (ISTA, 2020).

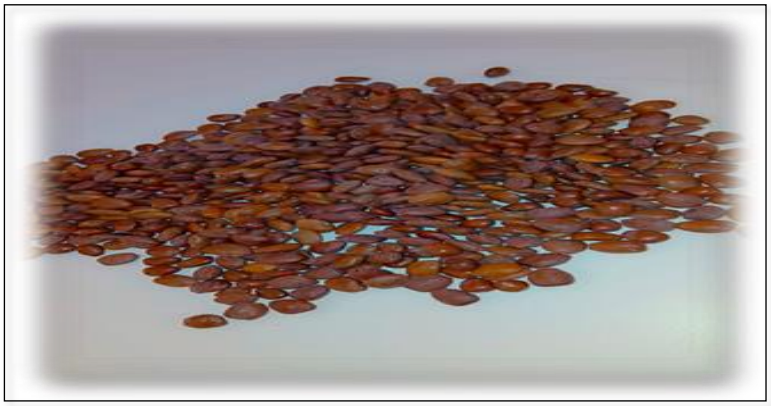

Figure 15*: Ceratonia siliqua L* seeds

III.3. Laboratory Equipment and Reagents

The following materials and reagents were used:

- Petri dishes (10 cm diameter)
- Filter paper
- Distilled water
- Sodium chloride (NaCl)
- Electronic balance
- 500 mL volumetric flask
- Beakers
- Graduated ruler
- Nail clipper (for mechanical scarification)
- Incubator
- Refrigerator (5 °C)
- Thermostatic incubator

III.4. Seed Preparation

III.4.1. Seed Selection and Cleaning

Seeds were initially rinsed with distilled water to remove surface impurities.

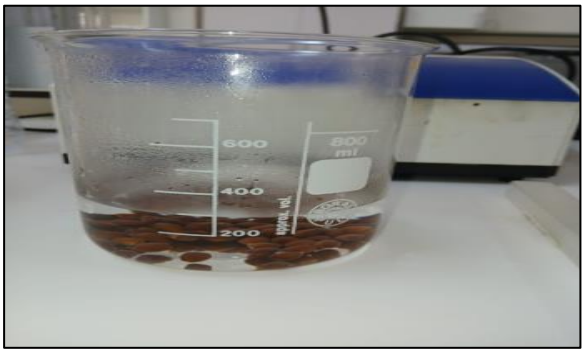

Figure 16: Soaking of *Ceratonia siliqua L* seeds in distilled water.

A portion of the seeds was disinfected by immersion in a 5% sodium hypochlorite solution for 15 minutes, followed by five successive rinses with sterile distilled water to eliminate any residual disinfectant.

Figure 17: Soaking of *Ceratonia siliqua L* seeds in sodium hypochlorite

Another portion of the seeds was left non-disinfected to evaluate the effect of this factor on germination performance.

Figure 18: Filtering of *Ceratonia siliqua L* seeds .

III.4.2. Dormancy-Breaking Treatments

Since *Ceratonia siliqua L.* seeds exhibit physical dormancy due to the impermeability of the seed coat, several treatments were applied to promote germination:

- **Mechanical scarification:** Mechanical scarification was performed using a nail clipper to slightly damage the seed coat, thereby facilitating water penetration. This method is widely recognized as effective in breaking physical dormancy in hard-coated seeds (Baskin & Baskin, 2014).

Figure 19: Partial scarfication of *Ceratonia siliqua L* seeds by cutting .

- **Thermal treatment:** Seeds were immersed in boiling water (100 °C) for 15 minutes.
- **Cold stratification:** Seeds were stored at 4–5 °C for a specified period to stimulate dormancy release.

Figure 20: Exposure of *Ceratonia siliqua L* seeds to cold stress at 4-5C°

- **Untreated seeds:** Used as experimental controls.

III.4.3. Imbibition

Scarified seeds were soaked in distilled water for 24 hours at room temperature to promote imbibition, which represents the first essential stage of germination (*Bewley et al., 2013)*

III.5. Preparation of Saline Solutions

To evaluate the effect of salt stress on germination, two sodium chloride NaCl concentrations were prepared:

- 100 mM

- 200 mM

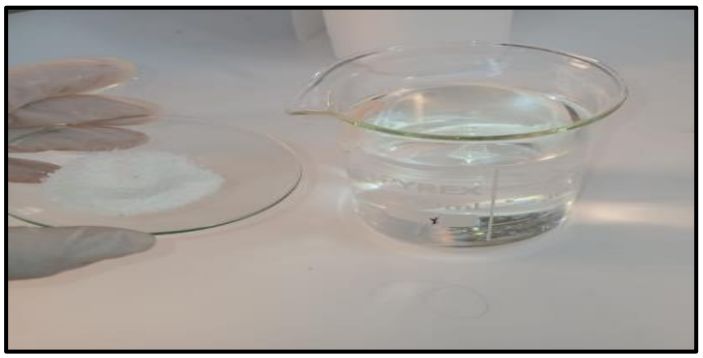

Figure 21: preparation of Ceratonia siliqua L NaCl solution

For the preparation of 500 mL solution:

- 2.92 g of NaCl were dissolved to obtain a 0.1 M solution

Figure 22: preparation of *Ceratonia siliqua L* NaCl (0.1 M) solution

- 5.84 g of NaCl were dissolved to obtain a 0.2 M solution

Figure 23: preparation of *Ceratonia siliqua L* NaCl (0.2 M) solution

The required quantities of NaCl were dissolved in distilled water and adjusted to the final volume using a volumetric flask. NaCl is widely used in salinity studies due to its osmotic and ionic effects on seed germination and early seedling development (Munns & Tester, 2008).

III.6. Experimental Design

A factorial experimental design was adopted, incorporating:

- Dormancy-breaking treatment
- Disinfection factor (disinfected / non-disinfected)
- NaCl concentration (0, 100, and 200 mM)

For each treatment, ten seeds were placed in a Petri dish between two layers of sterile filter paper moistened with 10 mL of the corresponding solution. Three replicates were performed for each treatment (Gomez & Gomez, 1984).

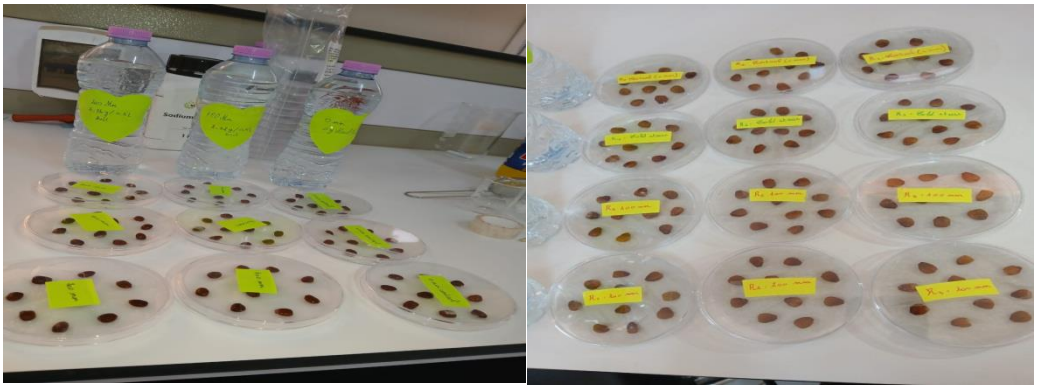

Figure 24: preparation of *Ceratonia siliqua.L* seeds in a petri dish

Petri dishes were carefully sealed to minimize evaporation and incubated in darkness at 27–28.

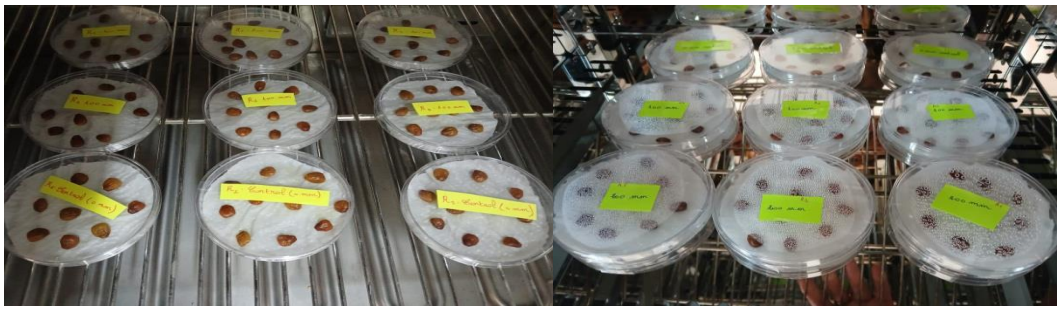

Figure 25: petri dishes were sealed to minimize evaporation and incubet in the dark at 27-28C°

An additional set of Petri dishes moistened with distilled water was incubated at 5 °C to evaluate the effect of low temperature (cold treatment).

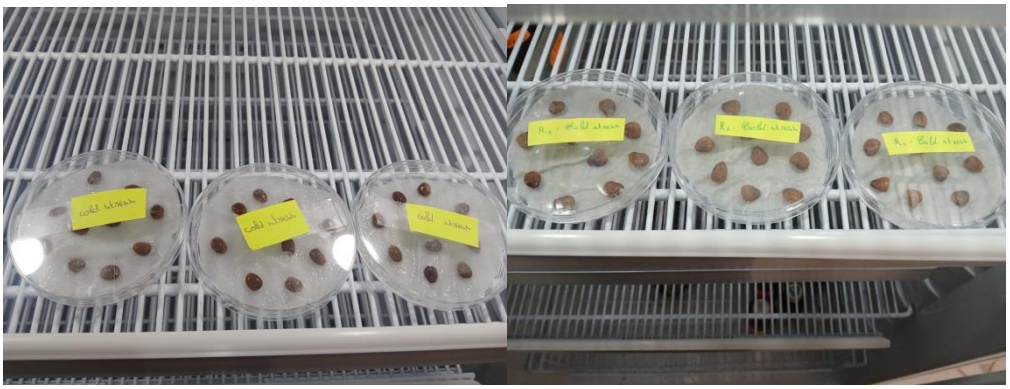

Figure 26: cold treatment of *ceratonia siliqua L* seeds at5C°

All treatments were maintained for 15days under dark conditions.

III.7. Germination Monitoring and Measured Parameters

The following germination parameters were evaluated:

A seed was considered germinated when the radicle reached at least 2 mm in length, according to international germination standards (ISTA, 2020).

The following parameters were recorded:

- Number of germinated seeds
- Germination percentage (%)
- Radicle length (cm)

III.7.1. Germination Percentage (GP)

$$\mathrm{GP}(\%) = \frac{\text{Total number of germinated seeds}}{\text{Total number of sown seeds}} \times 100$$

III.7.2. Germination Precocity

Defined as the percentage of seeds germinated within the first 24 hours after sowing.

III.7.3. Germination Rate

Germination rate was determined by monitoring the daily emergence of germinated seeds, reflecting the dynamics of the germination process over time.

III.7.4. Germination Energy

Represents the percentage of seeds that germinated during the first days of the experiment, indicating early vigor.

III.7.5. Radicle Length

Radicle length was measured using a graduated ruler and expressed in centimeters. Mean radicle length was calculated for each treatment (Bewley *et al*., 2013).

III.8. Statistical Analysis

Results were expressed as mean ± standard deviation (SD).

Analysis of variance (ANOVA) was performed to determine the effect of dormancy-breaking treatments and salt concentrations on germination parameters.

Statistical significance was considered at $p < 0.05$ (Gomez & Gomez, 1984).

Chapter IV

Results and Discussion

Before presenting the detailed analysis, it is essential to provide an overview of the germination behavior of *Ceratonia siliqua L.* seeds under different treatments. The effects of salinity (NaCl 0, 100, 200 mM) and cold treatment (5°C) on germination percentage and radicle length were evaluated over a 15-day period, following the procedures described in the Materials and Methods section. The following sections present the results along with scientific discussion and interpretation for each treatment.

IV.1. Germination Assays

IV.1.1. Number of Germinated Seeds

Seed germination was monitored over 15 days. Seeds were considered germinated when the radicle reached at least 2 mm.

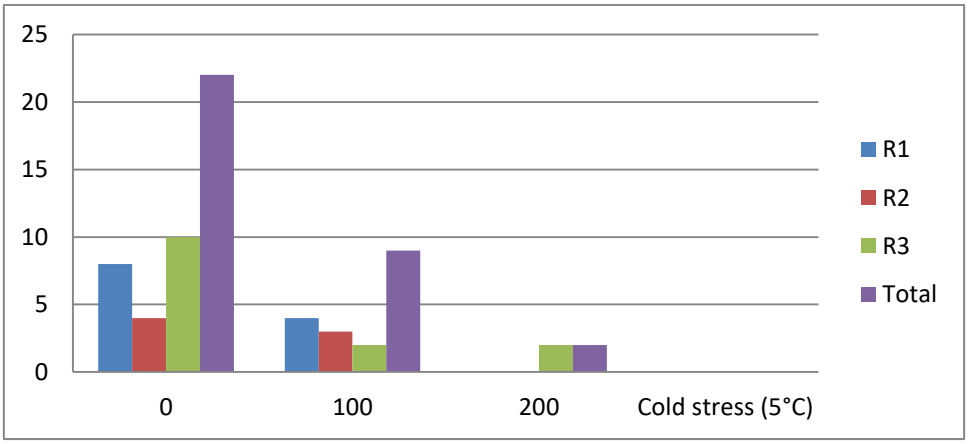

Figure 27: Bar chart representing the total number of germinated seeds for each treatment

The highest germination was observed under control conditions (0 mM NaCl), indicating optimal conditions for seedling establishment. Moderate salinity (100 mM NaCl) caused a noticeable reduction in germination, while high salinity (200 mM NaCl) severely inhibited germination. Seeds under cold stress (5°C) failed to germinate entirely. These results highlight the strong sensitivity of *C. siliqua* seeds to osmotic stress and low temperature during early germination.

IV.1.2. Germination Percentage

The germination percentage was calculated using the total number of germinated seeds per treatment

The germination percentage varied significantly depending on NaCl concentration and temperature conditions Under control conditions (0 mM NaCl), seeds showed the highest germination rate, reaching **73.30%**, indicating favorable conditions for seed germination.

However, the introduction of salt stress led to a marked decrease in germination. At **100 mM NaCl**, the germination percentage dropped sharply to approximately **30%**, representing more than a 50% reduction compared to the control. This decline became more pronounced at **200 mM NaCl**, where germination was severely inhibited, reaching only **6.66%**.

In contrast, seeds subjected to **cold stress (5°C)** exhibited a complete inhibition of germination (**0%**), indicating that low temperature had a more drastic effect than salinity under the experimental conditions.

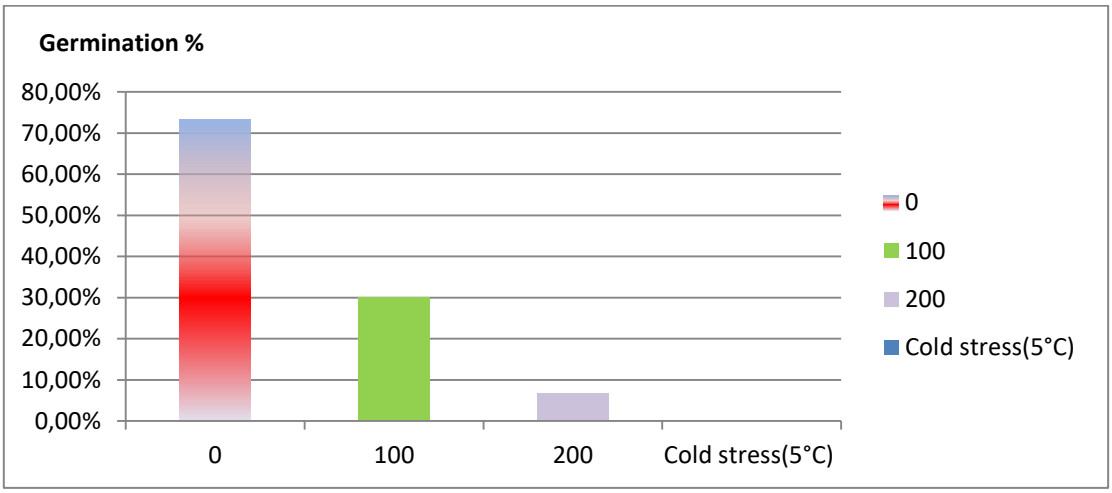

Figure 28: Germination percentage by NaCl concentration

The histogram clearly illustrates a progressive decrease in germination percentage with increasing NaCl concentration. The control treatment shows the tallest bar, reflecting optimal germination. In comparison, the bar corresponding to 100 mM NaCl is significantly lower, confirming the inhibitory effect of moderate salinity.

At 200 mM NaCl, the bar is almost negligible, indicating severe stress conditions that drastically limit seed germination. The absence of a bar for cold stress (5°C) highlights the complete suppression of germination under low temperature.

Overall, the graphical representation reveals a **negative correlation between salinity level and germination percentage**, with a steep downward trend as salt concentration increases.

The reduction in germination percentage under salt stress can be attributed to several physiological and biochemical factors. High salinity reduces water uptake by seeds due to increased osmotic pressure, thereby limiting the imbibition process essential for germination. Additionally, the accumulation of toxic ions such as Na^+ and Cl^- may disrupt cellular metabolism and enzyme activity, further inhibiting seed development.

At higher salt concentrations (200 mM), these effects become more severe, leading to almost complete inhibition of germination. This suggests that the studied seeds are highly sensitive to elevated salinity levels.

Cold stress (5°C), on the other hand, completely prevented germination, likely due to the inhibition of enzymatic activities and metabolic processes required for embryo growth. Low temperature may also reduce membrane fluidity and delay water absorption, thereby blocking the initiation of germination.

Both salinity and low temperature significantly reduce seed germination, with cold stress showing a more pronounced inhibitory effect . Germination success is highest under non-stress conditions, while increasing salinity results in a significant and progressive decline in germination percentage.

IV.2. Radicle Length

IV.2.1. Radicle Length under Different Treatments

Radicle elongation was measured at the end of the germination period
Radicle elongation was significantly affected by both salinity levels and cold stress .Under control conditions (0 mM NaCl), the radicle reached an average length of 2.75 cm, indicating normal post-germination growth and favorable environmental conditions.

At **100 mM NaCl**, a slight decrease in radicle length was observed (**2.65 cm**), suggesting that moderate salinity induces a mild inhibitory effect on root elongation without completely suppressing growth.

In contrast, at **200 mM NaCl**, radicle elongation was drastically reduced, with a mean length of only **0.33 cm**, reflecting severe inhibition.

Under **cold stress (5°C)**, no radicle growth was recorded (**0 cm**), indicating a total inhibition of root development under low temperature conditions.

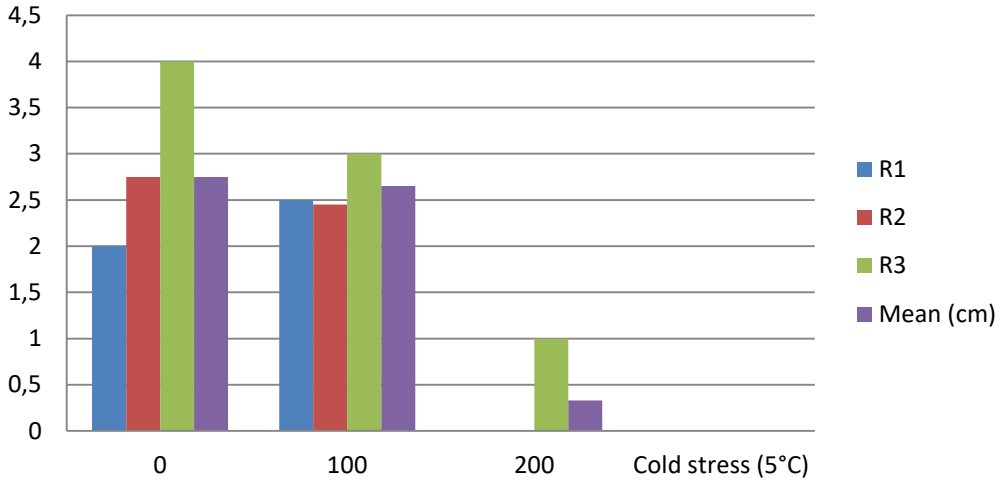

Figure 29: radicle length (cm) for each treatment NaCl concentration.

The graphical representation shows a clear decreasing trend in radicle length with increasing NaCl concentration. The control treatment exhibits the highest value, while a slight decline is observed at 100 mM NaCl.

At 200 mM NaCl, the graph shows a sharp drop in radicle length, confirming the strong inhibitory effect of high salinity. The absence of radicle growth under cold stress is represented by a zero value, highlighting the complete suppression of elongation.

Overall, the graph indicates a negative correlation between salinity level and radicle growth, with a progressive reduction as salt concentration increases.

Salt stress reduces radicle elongation mainly due to osmotic effects that limit water uptake and ionic toxicity (Na^+ and Cl^-) that disrupts cellular metabolism. As salinity increases, cell expansion and enzymatic activities involved in growth are progressively inhibited. At moderate salinity (100 mM), seeds still maintain some adaptive capacity, allowing limited radicle development. However, at high salinity (200 mM), severe stress almost completely stops root elongation. Cold stress (5°C) leads to total inhibition of radicle growth by reducing

enzyme activity and membrane fluidity. This also limits imbibition, preventing the physiological processes necessary for germination and root development.

IV.3. Stepwise Analysis According

The results showed that the effectiveness of treatments varied depending on their role in overcoming dormancy and supporting germination of *Ceratonia siliqua L.* Mechanical scarification was the most effective method, significantly improving germination by facilitating water uptake through the hard seed coat. In contrast, thermal and cold treatments had only moderate effects, indicating that temperature alone is insufficient to fully break physical dormancy.

Disinfection slightly enhanced germination, suggesting that microbial contamination may have a minor inhibitory effect, although it is not a determining factor.

Salinity stress (NaCl) negatively affected both germination percentage and radicle growth, with increasing concentrations leading to a clear decline. This effect is mainly due to osmotic stress and ion toxicity, which limit water absorption and disrupt metabolic activities.

Temperature also played a crucial role, as optimal germination occurred at 27–28°C, where enzymatic and physiological processes are more active. In contrast, low temperature (5°C) failed to promote germination and even inhibited seedling development.

Overall, these findings highlight that successful germination depends on effective dormancy-breaking methods, favorable temperature conditions, and the absence of environmental stresses such as salinity.

Chapter V

Interpretation

V.1 Physiological Response to Salinity (NaCl)

The experimental outcomes demonstrate a clear inverse relationship between increasing NaCl concentrations and *Ceratonia siliqua L* seed germination performance. Control seeds (0 mM NaCl) exhibited high germination rates and robust radicle elongation, whereas increasing salinity resulted in progressive inhibition. At 100 mM NaCl, germination decreased significantly, and at 200 mM NaCl, both germination percentage and radicle growth were severely reduced.

This pattern can be largely explained through **osmotic stress**: high NaCl concentrations lower the external water potential, creating a condition akin to physiological drought. This limits water uptake during seed imbibition, a critical step for activating metabolic processes and enzymes such as **α-amylase**, which are essential for mobilizing stored reserves and initiating radicle protrusion (Bewley *et al.*, 2013). Without adequate water absorption, seeds fail to activate these critical biochemical pathways, thus reducing germination success.

Furthermore, **ionic toxicity** contributes to the observed decline. Accumulation of Na^+ and Cl^- in embryonic tissues disrupts internal ionic balance, inhibits enzyme activities, and can lead to oxidative stress that damages cellular structures and meristematic regions, thereby arresting cell division and elongation (Munns & Tester, 2008; Lahbib, Hamza, & Ouerghi, 2018).

V.2 Thermal Constraints and Cold Stress

The complete inhibition of germination at 5°C indicates that low temperature acts as a more stringent physiological constraint than salinity in this species. At low temperatures, membrane lipid mobility is reduced, resulting in impaired solute transport and inactivation of key metabolic enzymes, including those responsible for respiration and ATP synthesis (Pérez-García, 2009). Consequently, the embryo cannot generate sufficient metabolic energy to support radicle emergence.

From an ecological perspective, this response reflects **thermo-dormancy**, an adaptive strategy observed in Mediterranean species such as *Ceratonia siliqua L* This mechanism prevents seed germination during cold periods when seedling survival is unlikely, ensuring that germination occurs under more favorable thermal conditions (Batlle & Tous, 1997).

V.3 Comparative Interpretation with Scientific Literature

V.3.1 Studies Consistent with the Present Results

Several studies align with the results obtained in this research:

1. ***Sbihi, Bouhaouel, & Arroussi* (*2019*)** reported that NaCl concentrations above 150 mM sharply reduce *Ceratonia siliqua L* seed germination, mirroring the near-complete inhibition observed at 200 mM in the current study..
2. ***Lahbib et al.* (*2018*)** found that high salinity disrupts ionic balance, particularly the K^+/Na^+ ratio, resulting in reduced radicle growth — consistent with the stunted radicle lengths measured under saline conditions in our work.
3. ***Parida & Das* (*2005*)** showed that salinity stress significantly decreases germination and early seedling growth in many leguminous plants, supporting the notion that osmotic and ionic stress are key limiting factors in seedling establishment.

V.3.2 Studies Providing Complementary or Contrasting Perspectives

While the general inhibitory effects of salinity and low temperature are widely supported, some studies offer complementary insights:

1. ***Tuna et al.* (*2008*)** demonstrated that exogenous application of gibberellic acid (GA_3) can partially mitigate salinity stress, suggesting that hormonal regulation may play a role in enhancing germination under saline conditions.

1. ***Otsamo, Eriksson, & Väisänen* (*1996*)** showed that cold stratification (a short period of cold followed by warmer conditions) can improve germination uniformity in Mediterranean legumes, indicating that the duration and timing of cold exposure affect germination responses.
2. ***Pérez-García* (*2009*)** emphasized the strict dependence of *Ceratonia siliqua L* seed germination on temperature, identifying optimal germination between 20–30°C and complete inhibition below 10°C, which aligns with the absence of germination at 5°C in the present study.

V.4 Implications and Synthesis

The synthesis of experimental results and comparative literature highlights that successful germination of *Ceratonia siliqua L* is a multifactorial process influenced by both physical pretreatments and environmental stresses. While mechanical scarification is essential to overcome physical dormancy, optimal germination requires favorable environmental conditions, particularly moderate temperatures and low salinity levels.

These findings have practical implications for propagation and reforestation programs. To maximize germination success and seedling vigor, seeds should be germinated in controlled environments with temperatures around 27°C and using low-salinity water. Additionally, the use of hormonal priming or controlled cold stratification could be explored as strategies to improve germination under sub-optimal conditions.

General Conclusion

Gneral conclusion

This study aimed to evaluate the effect of different sodium chloride (NaCl) concentrations on the germination of *Ceratonia siliqua L.* seeds, as well as on early seedling development, particularly radicle growth. The experiment also investigated the impact of low temperature stress (5 °C) and different dormancy-breaking treatments on germination performance.

The results clearly demonstrated that the germination of *Ceratonia siliqua L* seeds is highly dependent on environmental conditions. Under optimal conditions (0 mM NaCl and a temperature of 27–28 °C), seeds showed a high germination rate and normal radicle elongation. In contrast, increasing NaCl concentrations led to a progressive and significant reduction in both germination percentage and radicle growth.

Salinity stress mainly affects seed germination through osmotic effects, which reduce water uptake during imbibition, and through ionic toxicity caused by the accumulation of Na^+ and Cl^- ions, which disrupt essential metabolic and enzymatic processes. At high salinity levels (200 mM NaCl), germination was almost completely inhibited.

Temperature plays a fundamental role in seed germination, as it directly controls metabolic activity and enzymatic reactions required for seed development. In this study, low temperature (5 °C) completely inhibited germination, confirming that *Ceratonia siliqua* seeds require optimal thermal conditions for successful germination.

In addition, low temperature (5°C) resulted in a complete inhibition of germination, indicating that *Ceratonia siliqua* seeds require moderate temperatures for the activation of metabolic and enzymatic activities necessary for germination and seedling development.

Regarding dormancy-breaking treatments, mechanical scarification proved to be the most effective method, as it significantly improved germination by facilitating water penetration through the hard seed coat. Thermal treatment and cold stratification showed only limited effects.

earl, the findings of this study highlight that the successful germination of *Ceratonia siliqua L* depends on three key factors: effective dormancy breaking, optimal temperature conditions, and low salinity levels. Among these factors, salinity represents the most critical limiting stress affecting both germination and early seedling growth.

Finally, these results provide valuable insights for the propagation and cultivation of *Ceratonia siliqua L* trees, particularly in arid and semi-arid regions. They also contribute to a

better understanding of the physiological responses of *C. siliqua L* under abiotic stress conditions, which may support future reforestation and land rehabilitation programs.

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