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Theme

Effect of salinity on germination of common bean (*Phaseolus vulgaris)*

Supported on, ……..

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بسم الله الرحمن الرحيم
١٤٣٨

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DEDICATION

I dedicate this humble work to all the individuals whom I hold dear, and in particular:

*To my beloved **parents**, for their constant love and unwavering support. I owe all my successes, all my happiness, and all my joys to you. I am immensely grateful and proud to have you by my side.*

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Dedication

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LIST OF ABBREVIATIONS:

PLA/DAD1	phospholipase
A1/	defective in anther dehiscence 1;
LOX	13-lipoxyigenase;
AOS	allene oxide synthase;
AOC	allene oxide cyclase;
OPR/DDE1	OPDA reductase/delayed dehiscence 1;
OPCL	OPC-8:0 CoA ligase;
ACX	acyl-CoA oxidase;
JAR1	jasmonate resistant 1/jasmonate–amidosynthetase.
13-HOPT	13-hydroperoxylinoleic acid;
12,13-EOT	12,13-epoxyoctadecatrienoic acid;
OPDA	(9S,13S)-12-oxo-phytodienoic acid;
OPC-8:0	3-oxo-2(cis-2'-pentenyl)-cyclopentane-1-octanoic acid;
JA-Ile	jasmonoyl-L-isoleucine;
12OH-JA-Ile	12-hydroxy-JA-Ile.
GGPP	geranylgeranyldiphosphate,
CPS	copalyldiphosphate synthase,
CDP	copalyldiphosphate,
KS	kaurene synthase,
KO	kaurene oxidase,
KAO	ent-kaurenoic acid oxidase,
R.E	endoplasmic reticulum
GA	Gibberellin

FAOSTAT.Production Statistics of the Food Agriculture Organization of the United States.

ABSTRACT

Salinity is considered one of the abiotic stresses that significantly impact crop productivity worldwide. The bean is one of these sensitive plants, resulting in low yields in saline soils. Our study focused on this species, examining three levels of salinity (0meq, 150meq, and 250meq). All measured parameters, such as germination rate and amylase activity, showed low values with increasing salinity levels.However, we found that the exogenous application of gibberellin and salicylic acid improved the biochemical status of bean seeds. These results confirm, on the one hand, the negative effect of salt stress, and on the other hand, the importance of biostimulants and their impact in challenging germination conditions. Finally, germination plays a decisive role in the subsequent development of crops, underscoring the crucial importance of boosting this phase with biostimulants in the presence of salinity stress.

Keywords: bean, salt stress, germination, gibberellin, salicylic acid

Résumé

La salinité est considérée comme l'un des stress abiotiques ayant un impact significatif sur la productivité des cultures dans le monde. L'haricot est l'une de ces plantes sensibles, ce qui se traduit par de faibles rendements sur les terres salines. Notre étude s'est concentrée sur cette espèce en examinant trois niveaux de salinité (0meq, 150meq et 250meq). Tous les paramètres mesurés, tels que le taux de germination et l'activité amylasique, ont montré des valeurs faibles avec l'augmentation du niveau de salinité. Cependant, nous avons constaté que l'application exogène de gibbérelline et d'acide salicylique a amélioré le statut biochimique des graines d'haricot. Ces résultats confirment d'une part l'effet négatif du stress salin, et d'autre part, l'importance des biostimulants et leur impact dans des conditions difficiles de germination. Enfin, la germination joue un rôle déterminant dans le développement ultérieur des cultures, soulignant ainsi l'importance cruciale de stimuler cette phase avec des biostimulants en cas de stress salin.

Mots-clés: haricot, stress salin, germination, gibbérelline, acidesalicylique.

ملخص

يعتبر التأثير الملحي أحد الإجهادات البيئية التي تؤثر بشكل كبير على إنتاجية المحاصيل في جميع أنحاء العالم. الفاصوليا هي إحدى النباتات الحساسة لهذه الظاهرة، مما يؤدي إلى إنتاج ضعيف على التربة الملحية. تركزت دراستنا على هذا النوع من النباتات من خلال فحص ثلاث مستويات ملحية (0 ميكرومول، 150 ميكرومول، و250 ميكرومول). أظهرت جميع المؤشرات المقاسة، مثل معدل الانبات ونشاط الأميليز، قيمًا منخفضة مع زيادة مستوى التأثير الملحي. ومع ذلك، لوحظ أن تطبيق الجيبيريلين وحمض الساليسيليك بشكل خارجي قد أحسن من الحالة الكيميائية لبذور الفاصوليا. تؤكد هذه النتائج، من جهة، التأثير السلبي للملوحة ومن جهة أخرى، أهمية المحفزات الحيوية وتأثيرها في ظروف صعبة لعملية الانبات. في النهاية، تلعب عملية الانبات دورًا حاسمًا في التنمية المستقبلية للمحاصيل، مما يؤكد أهمية بروز هذه المرحلة وتعزيزها باستخدام المحفزات الحيوية في حالات التأثير الملحي.

الكلمات الرئيسية: الفاصوليا، التأثير الملحي، الانبات، الجيبيريلين، حمض الساليسيليك

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GENERAL INTRODUCTION

INTRODUCTION:

The common bean, scientifically known as *Phaseolus vulgaris* L., is globally acknowledged as one of the most popular leguminous crops (Castro-Guerrero et al., 2016). Its outstanding nutritional characteristics have elevated it to the status of a dietary staple in Latin America, where it forms a substantial portion of people's food consumption (Torres et al., 2017). Depending on the specific landrace, the common bean exhibits a protein content ranging from approximately 16% to 33% (Luna-Vital et al., 2015). Another advantage of this legume is its eco-friendliness, attributed to its symbiotic relationship with nitrogen-fixing bacteria, which reduces the dependency on synthetic fertilizers and promotes sustainable agricultural practices (Castro-Guerrero et al., 2016).

Approximately 8,000 years ago, the domestication of common beans took place in Mexico/Central America and South America, resulting in subsequent adaptations to Mediterranean environments and the emergence of two prominent gene pools known as Mesoamerican and Andean (Castro-Guerrero et al., 2016). Among the nearly 200 recognized common bean landraces in Chile, notable types include Tortola, Coscorron, Manteca, Bayo, Araucano, Peumo, and Sapito. These varieties are considered representative of the local bean types(Bascur and Tay, 2005).

Throughout time, traditional bean varieties have been substituted with modern resistant ones to cope with the diverse biotic and abiotic stresses encountered by beans. Nevertheless, the continuous occurrences of global warming and climate change have amplified the difficulties confronted by agricultural soils in Mediterranean regions. The increasing temperatures, drought conditions, and soil salinization have notable repercussions on crop growth and productivity on a global scale, presenting significant abiotic stress factors (Jacobsen et al., 2012). Previous studies have demonstrated that salinity and drought often occur concurrently, leading to more severe consequences, including impaired germination of crops (Dawood et al., 2021).

Salinity represents a significant threat to agriculture in arid and semi-arid regions,and is a key factor limiting global food production (Ahmed et al., 2020; Tolay, 2021). The high evaporation rates and desertification in these areas lead to rapid soil and water salinization (Bensidhoum&Nabti, 2021), while even trace amounts of NaClinirrigation water can exacerbate the problem of soil salinity, which is becoming increasingly prevalent (Chandel et al., 2021; Neji et al., 2021; Tolay, 2021).

For this reason plants have developed various strategies to regulate seed germination and ensure successful seedling growth under different environmental conditions. The physical characteristics of seed coverings contribute to the mechanisms of germination, while the physiological state of the embryo itself is also a crucial determinant of seed germination (Bewley et al. 2013). Seed dormancy, which suspends germination under otherwise favorable conditions, has played a critical role in the survival of seed plants. In order to obtain a thorough comprehension of seed germination and dormancy, it is essential to explore the modifications that occur in seed coverings during imbibition, which facilitate the emergence of the radicle. Additionally, understanding the molecular and biochemical processes that inhibit embryo growth during dormancy is crucial (Nonogaki 2014; Nonogaki 2017).

Additionally, gibberellins, a class of plant hormones, play multifunctional roles in germination, flowering, sex determination, and seed and fruit development (Procházka et al., 1998). Gibberellic acid, a type of gibberellin, is commonly used to overcome seed dormancy in many plant species (Castro-Camba et al., 2022).

The objective of our study is to assess the impact of salinity on common bean and examine its response to this stress by utilizing gibberelins.

PART I
SYNTHESIS BIBLIOGRAPHIC

CHAPTER I

THE COMMON BEAN

(*PHASEOLUS VULGARIS*)

I.1. Origin and geographical distribution:

The common bean, *Phaseolus vulgaris* L., was domesticated in Central America and South America over 9,700 years ago. Its domestication occurred independently in Mexico and Guatemala on one hand, and in Peru and neighboring countries on the other hand. Wild ecotypes with small seeds are found in northern Argentina and Central America (GENTRY, 1969).

Dry beans were introduced and planted in Europe in the 16th century, and its cultivation quickly spread to Mediterranean and subtropical regions (PERON, 2006).

The common bean is primarily produced in Latin America and Africa. It is widespread, especially in the Amazon region of Brazil, the Andes Mountains, and Central America. In Africa, it is mainly produced in Central and Eastern Africa (NYABYENDA, 2005).

This vegetable was introduced to Europe in the early 16th century but remained primarily consumed as grains for many years. In the 18th century, Italians began eating the immature pods of beans as a vegetable (Baudouin et al., 2001).

I.2DESCRIPTION OF THE PLANT:

I.2.1 MORPHOLOGICAL AND BOTANICAL CHARACTERS OF THE BEAN:

I.2.1.1 UNDERGROUND PART

I.2.1.1.1 Roots

The common bean possesses a root system with multiple lateral and adventitious ramifications (NYABYENDA, 2005). The bean root gradually forms after the germination stage, and the initial root system of the bean develops from the radicle of the embryo, which becomes the primary root (Chaux and Foury, 1994). Moreover, the main root can be easily hindered by soil obstacles. Lateral roots have a development that can surpass that of the main root (Guignard, 1998). The taproot system, which can reach a depth of up to 1.2 meters, has the largest number of roots between 0.20 to 0.25 meters deep (Barreto, 1983).

I.2.1.2 AERIAL PART

I.2.1.2.a-Stems

The tall stems of common beans can reach lengths of 2 to 3 meters, while the ones with short climbing stems hardly exceed 30 to 40 centimeters in length. Common beans with such stems are referred to as dwarf beans (Dupont and Guignard, 1989).

I.2.1.2.b-Leaves

According to GALLAS and BENNFORT (1992), the first two leaves are simple and attach to the stem face to face, while all the subsequent leaves are trifoliolate, arranged alternately. They are usually oval-shaped, measuring between 7.5 and 14 centimeters in length and 5.5 to 10 centimeters in width. The lateral leaflets are asymmetrical, while the central one is symmetrical.

The leaf of the green bean is entirely occupied by three veins from the base. This plant has two types of leaves: at the second node, two of the first leaves called primary leaves are formed. The typical bean leaves emerge from the third node (Gallais and Bennfort, 1992).

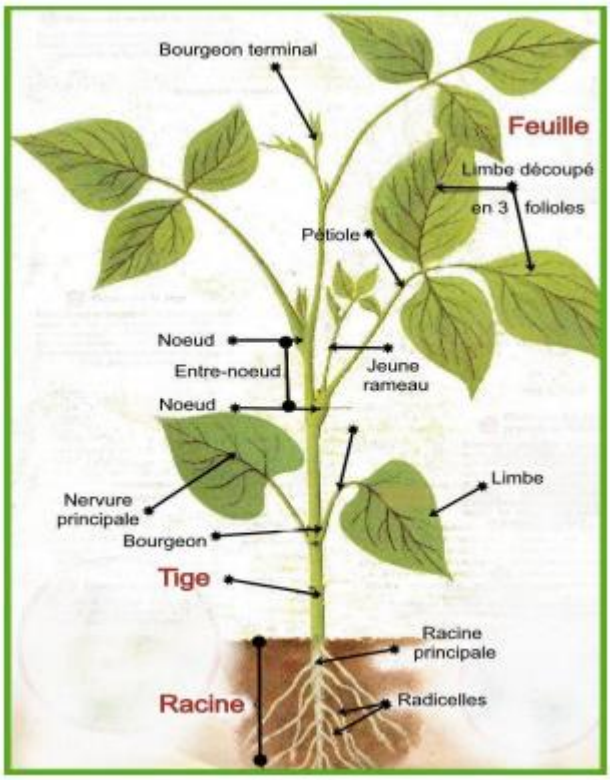


Figure n° 1: Description of the Bean plant

I.2.1.2.c Flowers

The bean flowers exhibit the characteristic architecture of the Fabaceae family. They are borne in short axillary clusters, ranging from 4 to 10 flowers. The papilionaceous corolla features a greenish to carmine standard, two white to lilac wings, and a similarly colored keel.

In the case of beans, the flower remains naturally closed. Exceptionally, certain hymenopterans (such as bumblebees) manage to force their way through the corolla barrier, thus allowing the introduction of foreign pollen into the flower (CHAUX and FOURY, 1994). (Figure: 02)

Figure n° 2: Common bean flower“Phaseolus vulgaris by Dingilingi 2020/09/29

I.2.1.2.4 Fruits

According to Solen (Hubert 1978), the beans have elongated pods that are typically straight, varying in length and ending in a point. Their width ranges from 8 to 25 mm. On average, they contain 4 to 8 seeds. The walls of the pod, called the husk, may have more or less developed woody vascular bundles (Goust and Seignobos, 1998). Figure 03

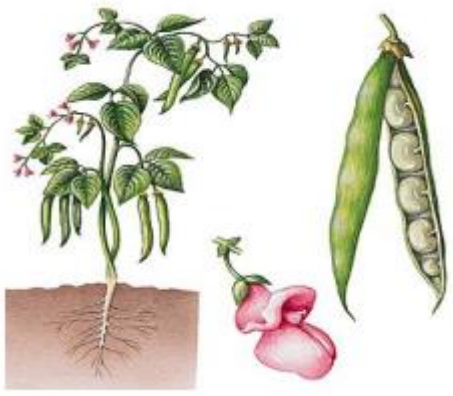


Figure n° 3: common bean fruit by Chantal Beaumont - Archives Larousse

I.2.1.2.5 Seeds

The seeds are kidney-shaped, rounded to elongated ovals. They are rich in starch and protein. Resembling a kidney, they have a scar or hilum on the concave side (Chaux and Foury, 1994). The seed coat can be black, white, or covered in various shades of yellow, brown, red, or pink, depending on the variety (Peron, 2006).

I.3 CLASSIFICATION:

The genus Phaseolus belongs to the subtribePhaseolinae, tribe Phaseoleae, family Fabaceae, and order Fabales. According to the APG (2003), the classification of beans is as follows:

I.3.A.TRADITIONALCLASSIFICAT:

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Fabaceae

Genus: Phaseolus

I.04 BEAN DEVELOPMENT CYCLE:

It begins with the formation of a main zygote and an accessory zygote resulting from the double fertilization of the embryo sac, which is enclosed within the ovule. Protected by the pistil of the flower, the accessory zygote will develop into a nutritive tissue called endosperm, while the main zygote gives rise to a new plant. The main zygote undergoes numerous mitotic divisions to form an embryo, which consists of two foliar lobes called cotyledons filled with reserves. The mature embryo is protected within the dormant mature seed inside a fruit called a "pod."

When conditions are favorable and dormancy is lifted, the seed becomes active and germinates. The radicle pierces the seed coat and grows into the soil, while the hypocotyl grows towards the sky, lifting the cotyledons above the ground towards the light. The seedling becomes autotrophic and grows until it reaches the adult stage, where it flowers. The adult plant exhibits a vegetative apparatus composed of an underground root system and an aboveground shoot system (stem and leaves) developed in the aerial environment (Meyer et al.,2008

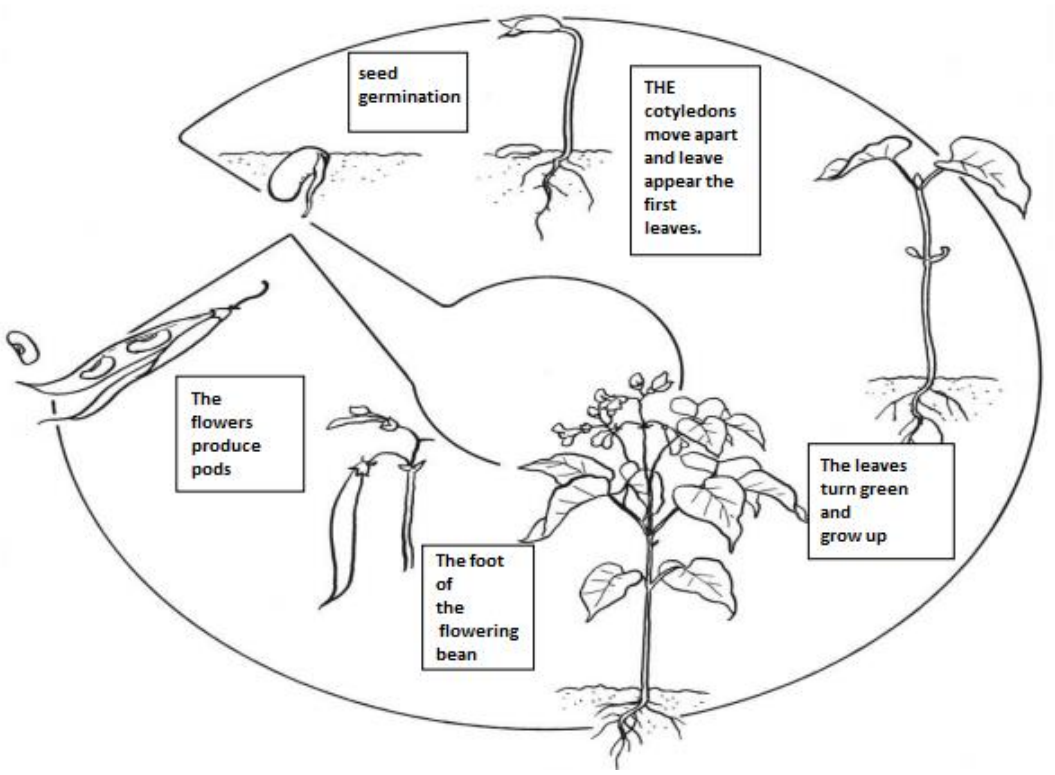


Figure n° 4: Phaseolus vulgaris crop development cycle (DIAW, 2002)

I.5 AGRO-MORPHOLOGICAL CHARACTERISTICS:

Beans are annual herbaceous plants with determinate or indeterminate growth (LAUMONNIER, 1979). Upon germination, the plant usually has taproots, but soon after, long adventitious roots measuring 10 to 15 cm develop along the entire main root. The flowers are borne in axillary and terminal clusters. They are zygomorphic, consisting of two keel petals, two wing petals, and one standard petal arranged externally. The color of the flower is generally independent of the seed color, but there is a known association between specific flower types and seed color (DIAW, 2002). In beans, the duration of the developmental stages varies considerably depending on the variety and environmental conditions (ADAMS et al., 1985).

I.6 IMPORTANCE OF BEAN CULTIVATION:

Bean cultivation is important for human consumption, with the pods or seeds being consumed fresh or as dry seeds, as well as for animal feed (crop residues such as stems and pods). Indeed, beans constitute a staple food for nearly 500 million people due to their high protein content (approximately 25%) (PUJOLA et al., 2007). From an agronomic perspective, as a legume, beans can be integrated into organic production systems that utilize bio-fertilization. They are used in rotations and intercropping systems with other crops, particularly cereals, to ensure the optimal utilization of nitrogen resources (CANADO et al., 2003).

I.7PRODUCTION OF BEEN IN ALGERIA:

In Algeria, beans are the predominant food legume cultivated [FAOSTAT (accessed on 3 June 2021)] , with a significant annual production of 55,272 tons from a total cultivated area of 41,451 hectares [Food and Agriculture Organization(accessed on 2 June 2021)]. These beans are commonly used in Algerian cuisine, either in dry or fresh form, and are primarily consumed for homemade meals and subsistence purpose

CHAPTER II
SALT STRESS

II .1DEFINITION OF STRESS:

Saline stress is defined as an excessive concentration of salt. The term saline stress mainly applies to an excess of ions, particularly Na+ and Cl- (Hopkins, 2003). Soil salinity is one of the primary abiotic stresses that limit the growth of cultivated plants. This salinity can be natural or induced by agricultural activities such as irrigation or the use of certain types of fertilizers (Jabnoune, 2008). Currently, out of the 1.5 billion hectares of cultivated land worldwide, approximately 77 million hectares (5%) are affected by excessive salt content. This number continues to increase from year to year due to the poor quality of irrigation water (R'him et al., 2013).

II.2.THE DIFFERENT ABIOTIC STRESSES:

II.2.1 WATER STRESS:

Water deficit poses a permanent threat to plant survival, but many plants produce morphological and physiological modifications that allow them to survive in regions with low rainfall and low soil moisture content (Hopkins, 2003).

II.2.2 HEAT STRESS:

Heat stress is often defined as temperatures that are high or low enough for a sufficient amount of time to irreversibly damage plant function or development. Plants can be damaged in various ways, either by high or low temperatures during the day or night, by hot or cold air, or by high soil temperatures. The thermal stress is a complex function that varies depending on the intensity (degree of temperature), duration, and rates of temperature increase or decrease (OUKARROUM, 2007).

II.2.3 SALT STRESS:

The alt concentration in a plant environment varies greatly and can be either insufficient or excessive. Although low salt concentrations can be a stress factor, a deficiency in salt typically presents as a nutritional problem. In fact, the term salt stress mainly applies to an excess of ions, particularly but not exclusively Na+ and Cl-. There are vast areas on the Earth surface where high salinity is a natural part of the environment (KABAR, 1986).

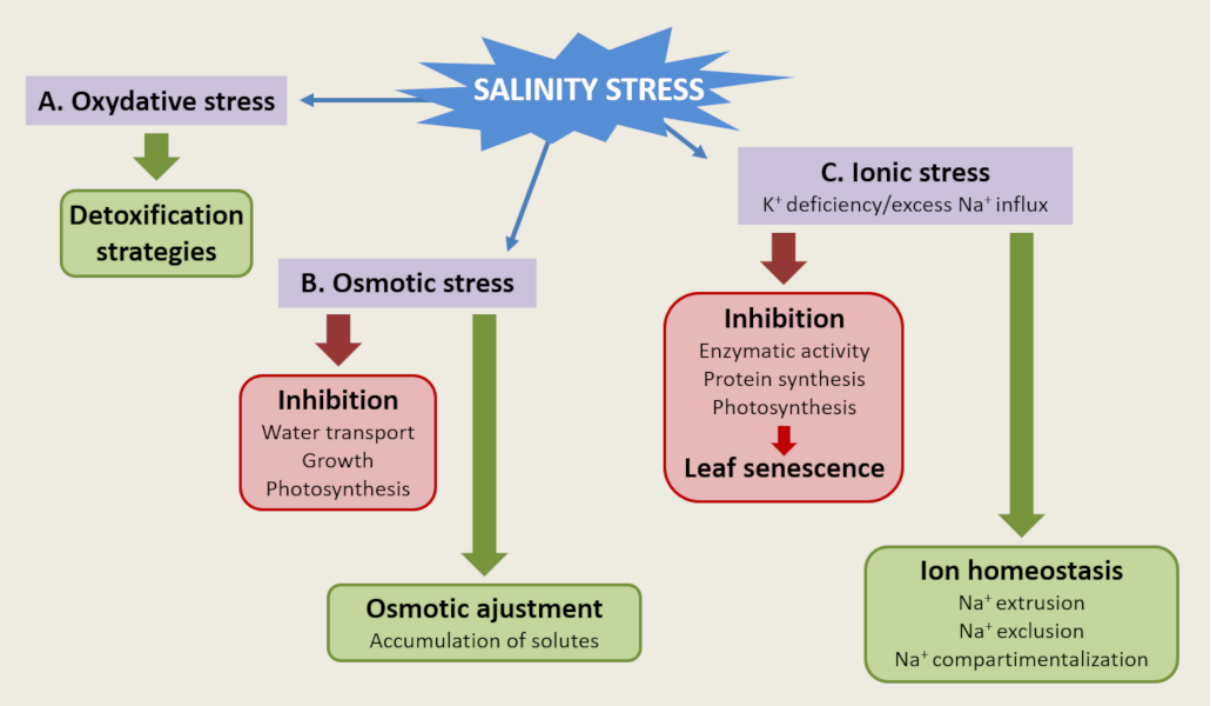


Figure n° 5: Deleterious effects related to salinity stress toxicity and the cellular response set up to ensure tolerance. In halophilic plants, excess salt in the soil causes ionic, oxidative and osmotic stress, which they must manage by implementing strategies to maintain (A) oxidative stress molecules at an acceptable level, (B) osmotic balance, and (C) ionic homeostasis. [Source: © EEnv diagram].

II.3. DEFINITION OF SALINITY:

The presence of excessive concentration of soluble salts in soil or the presence of abnormally high concentrations of sodium (Na+), calcium (Ca+2), and magnesium (Mg+2) in the form of chlorides, carbonates, or sulfates has been defined by several authors as soil salinity (Asloum, 1990). It is common in arid and semi-arid ecosystems and results from high water evaporation from the soil and irregular and insufficient rainfall (Taji et al., 2002). High soil salinity, primarily caused by sodium chloride, affects one-third of the irrigated land globally and is a significant limiting factor for plant production in arid regions. This highlights the need for strategies to mitigate the impact of soil salinity on agricultural productivity in these areas.(HASEGAWA et al, 1986 in: NDEYETHIORO,2000)

II.4. ORIGIN OF SALINITY:

Resulting from the salts formed during the weathering of rocks or natural external inputs (Bryssine, 1961). Meanwhile, 20% of salinized lands have an anthropogenic origin. This is known as secondary salinization, induced by human activity, particularly agricultural practices such as irrigation (FAO, 2008).

II.5. EFFECT OF SALINITY ON THE PLANT:

II.5.1. ON GRAIN GERMINATION:

During the phases of germination and emergence, most plants are more sensitive to salinity (Maillard, 2001). One of the factors inhibiting germination in the presence of salt is the variation in hormonal balance (UNGAR, 1978 and KABAR, 1986 in BOUCHOUKH, 2010). Despite having a high salt content in their tissues as adults, halophytes are not as salt-tolerant during the germination stage (BELKHODJA and BIDAI, 2004). The germination stage is often limited by soil salinity and is more sensitive than other stages (BOUDA S and HADDIOUI, 2011).

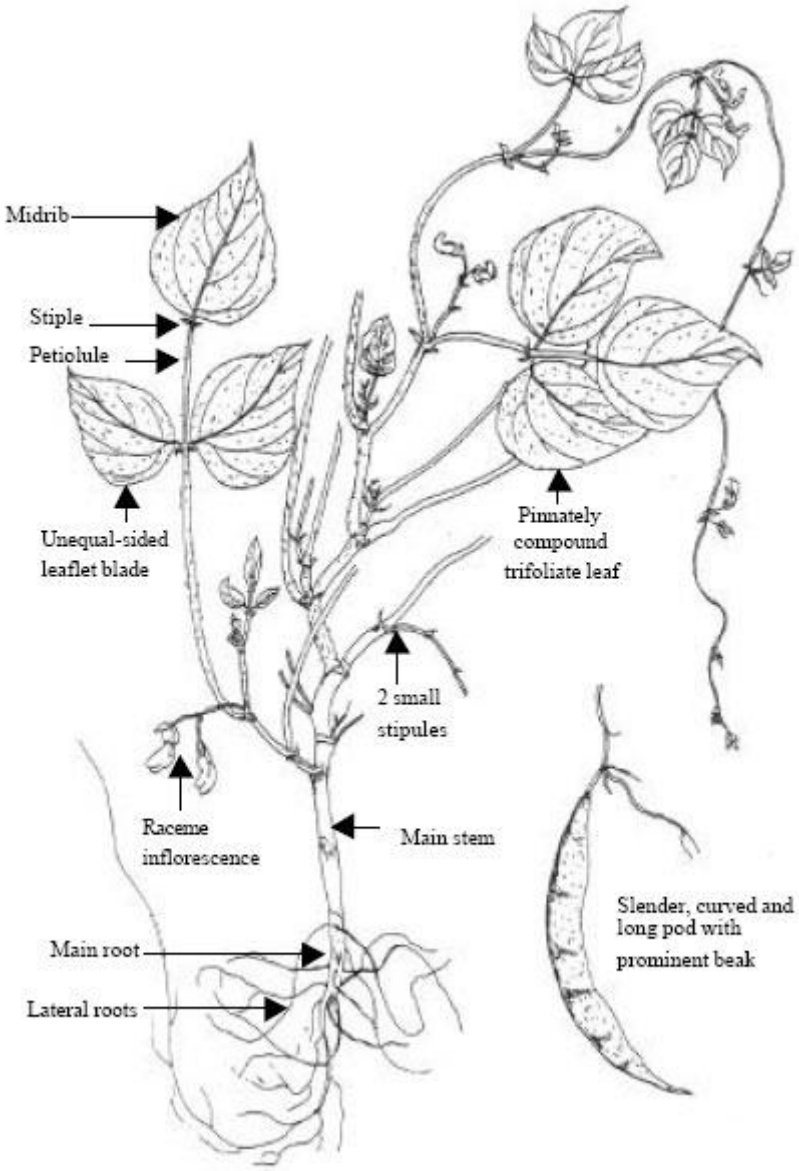


Figure n° 6: Schematic representation of *Phaseolus vulgaris L*. plant, 8 weeks old, showing vegetative and reproductive structures

II.5.2. ON GROWTH AND DEVELOPMENT:

According to Wang and Nil (2000), when plants are subjected to saline stress, their immediate response is a reduction in the rate of expansion of their leaf surface. If the salt concentration increases, this reduction can even lead to a complete stop in expansion.

In general, when plants are exposed to saline stress, it begins with the exposure of their roots to this stress. The salinity present in the soil can affect the availability of nutrients and water, creating osmotic stress. This osmotic stress can induce physiological drought, which results in an overall reduction in plant growth, as highlighted by Munns and Tester (2008).

II.5.3.ON WATER IN THE PLANT:

High salt concentrations in soil are first perceived by plants as the amount of water available has plummeted. This requires proper osmoregulation, maintain the cellularwater potential below that of the extracellular medium, and the one on the ground. On the one hand, this phenomenon ensures the continuous absorption of water from the soil, on the other hand, intracellular water retention and maintenance of swelling. when adjusting insufficient osmotic pressure, water tends to leave the cells, resulting in a deficit loss of water and swelling.(Niu et al., 1995 ; Bohnert et Shen, 1999 ; Hasegawa et al., 2000).

II. 5.4.ON PLANT PHYSIOLOGICAL PROCESSES:

Excess salt in the protoplasm can cause changes in the ion balance. Induces low energy production through phosphorylation and photorespiratory reactions. Nitrogen assimilation and many metabolic pathways are disrupted. If the concentration in the saddle exceeds the plant tolerance level, there will be dysfunction at the level of photosynthesis due to the action of salts in the chloroplast stroma disrupting the transport of electrons. Glycolysis and the Krebs cycle are also affected. Absorption of minerals such as potassium, nitrate or calcium is also reduced .Plants then show signs of productive stress Destruction of anthocyanins or chlorophyll. If growth is stimulated by a moderate supply of salt in some halophytes, this phenomenon is still somewhat limited tolerant. Extreme stress can lead to dwarfism and growth inhibition. The leaves become hardened even before they finish growing and developing, and the whole organism risks wilting quickly.(Ben Hayyim et al, 1989 ; Speer et Kaiser, 1991).

II.6. ADAPT THE PLANT TO SALINITY:

Methods of acclimatizing plants to salinity can be divided into three parts: tolerance, acclimatization and resistance.

II.6.1. SALT RESISTANCE:

Plants have developed various mechanisms to cope with high salt concentrations in their environment. One common mechanism is the exclusion of sodium and chloride ions from the roots through the regulation of ion transporters such as SOS1 and NHX1. Additionally, plants may accumulate compatible solutes such as proline and glycine betaine, which can help maintain cellular functions under high salt conditions. Some plants also form specialized structures, such as salt glands or bladders, to excrete excess salt. Other strategies include the synthesis of specific proteins and enzymes that enable the plant to detoxify reactive oxygen species produced under salt stress.

These mechanisms have been extensively studied in many plant species, and recent research has focused on identifying salt tolerance genes and their functional roles.

II.6.2. TOLERANCE:

Plants growing under conditions of soil salinity experience physiological and biochemical disturbances (BEN NACEUR et al., 2001). The response of plant species to salt depends on the species itself, its variety, salt concentration, growing conditions, and plant development stage (MALLEK-MAALEJ et al., 1998).

II.6.3. ACCLIMATION:

Plant acclimation to salt environments varies among plant species. Adaptation in these environments is reflected in the extent of salt resistance (Farsha, 2001),

CHAPTER III
GERMINATION

III.1. DEFINITION OF GERMINATION:

Agreeing to Sergio. (2016), germination definded as the move from torpidity to dynamic life, and the seed assimilates 20% to 25% of its weight in water.Emergence is characterized by coleoptile bulge and seeding within the embryo Seed germination may be a characteristic wonder that happens when seeds are splashed in water beneath appropriate conditions of temperature, oxygen and haziness (Baumgartner and Emonet, 2007)

III.2. TYPES OF GERMINATION:

III.2.1. EPIGEAL GERMINATION:

The seed is lifted out of the ground as there's a fast increment within the stem which gives the hypocotyl pivot which lifts the two cotyledons out of the ground. The gemmule creates (after the radicle) and gives a verdant stem over the two cotyledons. The primary internode gives the epicotyl. The primary takes off, over the cotyledons are the primordial takes off (Ammari, 2011).

III.2.2 HYPOGEAL GERMINATION:

The seed remains within the ground, the stem does not create and the cotyledons stay within the ground (Ammari, 2011).

III.3.MEASUREMENT OF GERMINATION CAPACITY:

According to Bewley& Black (1994), the term germination is frequently used inaccurately and interchangeably with seedling growth, which actually begins after germination is complete. The overall germination process consists of three distinct stages: imbibition, activation, and intra-seminal growth, culminating in embryo protrusion (Labouriau 1983a). Determining the precise start and end points of each stage is challenging due to the involvement of molecular and cellular events within a complex, multicellular tissue. The reactivation of the process occurs gradually, further complicating the task. Consequently, instead of relying on molecular or cellular criteria, the beginning of the germination process is typically determined using macroscopic indicators, such as embryo protrusion. This criterion provides a certain level of consistency, as multiple individuals can refer to the same reference point. As aptly stated by Bewley& Black (1994),

III.3.1.SEED DORMANCY:

In many plants, seed germination is not immediate and requires a period of rest during which germination is inhibited by various mechanisms. Dormancy is an important stage in the life cycle of plants. It is a temporary state in which viable seeds cannot germinate even under favorable conditions, and is characterized by a virtual absence of metabolic activity and/or a virtual lack of development and growth (HILHORST and KOORNNEEF, 2007). Dormancy can be linked to the presence of inhibitors, the presence of photosensitive or chromoproteins, the impermeability of the seed coat to water or oxygen, and/or the mechanical resistance of the coat. It is an innate property defined by genetic and environmental factors during seed development. Dormancy corresponds to the inability of the seed to germinate even under favorable conditions (BEWLEY, 1997). Dormancy is acquired at the end of seed maturation.

III.3.2.TYPES OF DORMANCY:

There are two types of dormancy:

III.3.2.a-Primary dormancy:

It occurs during seed formation and is present at harvest. It is a state of deep rest that occurs under the influence of internal factors such as the tegument or embryo nature. The onset of primary dormancy is shown to be dependent on ABA. Overexpression of ABA biosynthesis enzymes promotes dormancy, while ABA-deficient seeds do not exhibit dormancy (NAMBARA and MARION-POLL, 2005; FINCHTEL-SAVAGE and LEUBNER-METZGER, 2006).

Tegumentary dormancy: The teguments normally protect the seeds, but in many cases, they can prevent germination by acting as a physical barrier (mechanical resistance, impermeability to water) or chemical barrier (trapping oxygen by phenolic compounds, presence of germination inhibitors in the teguments).

Morphological (embryonic) dormancy: Morphological dormancy is due to the presence of an "underdeveloped" embryo at the time of seed dispersal (BASKIN and BASKIN, 1998). Germination cannot occur until the embryo has completed its growth. Embryo dormancy involves other factors such as cotyledons and germination inhibitors, especially abscisic acid (ABA) (BEWLEY and BLACK, 1994). Among embryonic dormancies, photoblastic, scotoblastic, xeroblastic, and psychrolabilic dormancies can be distinguished (HELLER et al., 1990).

III.3.2.b-secondary dormancy:

There is a type of dormancy called secondary dormancy, which occurs after the harvest during storage when external factors such as temperature, oxygen, and light are unfavorable for seed preservation. If conditions are not conducive to germination and inhibition of dormancy, secondary dormancy automatically begins after the release of primary dormancy (FINCH-SAVAGE and LEUBNER-METZGER, 2006). ABA levels also seem to play a role in inducing secondary dormancy. For example, in Brassica napus, an increase in ABA concentration within the seed is associated with the induction of secondary dormancy (WENTAO et al., 2009). Dormancy is a complex process regulated by both endogenous signals within the seed and environmental factors. The balance of abscisic acid (ABA) and gibberellic acid (GA) hormones within the seed is a major regulator of dormancy, with ABA promoting dormancy and GA inhibiting it (MATILLA and MATILLA-VAZQUEZ, 2008).

III.3.3.DORMANCY BREAKING:

The breaking of dormancy, accomplished through various mechanisms involving complex interactions between the environment and internal factors (FINKELSTEIN et al., 2008), is characterized by an increase in GA biosynthesis and degradation of ABA (FINCH-SAVAGE and LEUBNER-METZGER, 2006) (Fig.7).

Several techniques, varying according to the species and nature of dormancy, are recommended for breaking dormancy before sowing or germination tests. Cold stratification (vernalization) or warm stratification (estivation), scarification (mechanical, chemical, or physical), removal of seed coats, and elimination of inhibitory substances are proposed methods (BACCHETTA et al., 2006).

The induction and breaking of dormancy (primary or secondary) are controlled by various mechanisms that include complex interactions between the environment and two main phytohormones: abscisic acid (ABA) and gibberellins such as gibberellic acid (GA3) (Fig.7).

ABA promotes the induction and maintenance of dormancy during embryonic maturation. This hormone can inhibit germination, and its accumulation is correlated with the onset of dormancy (HILHORST and KOORNNEEF, 2007). Gibberellins, on the other hand, are known to promote the process of dormancy release and germination (FINKELSTEIN et al., 2008) in several plant species.

These hormones stimulate germination by inducing hydrolytic enzymes that weaken tissue barriers such as endosperms and seed coats, by promoting the mobilization of seed storage reserves, and by stimulating embryo expansion.

Studies have proposed the hormonal balance theory, which states that seed dormancy and germination depend on the accumulation of ABA and GA. Environmental signals regulate this balance by modifying the expression of catabolic and biosynthetic enzymes (FINKELSTEIN et al., 2008).

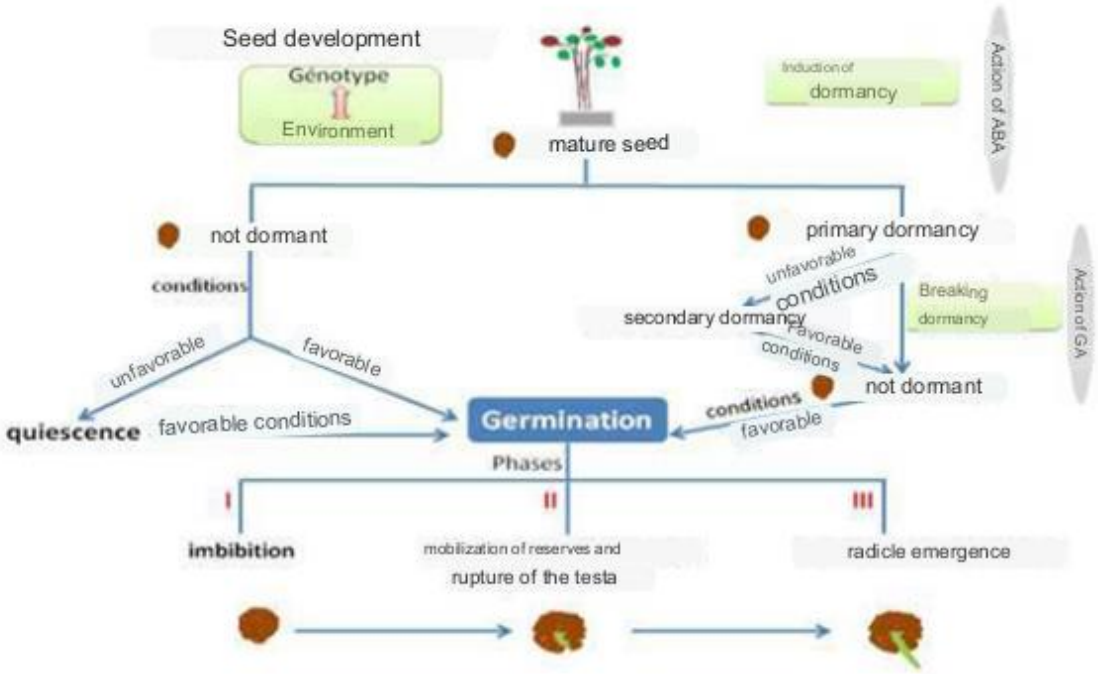


Figure n° 7:- Influence of environmental conditions on seed development, dormancy and germination. (N'DRI et al., 2011)

III.4.STARCH:

Among the family of carbohydrates, starch holds a unique position. It is found in the storage organs of numerous plants and is considered the most abundant natural polymer after cellulose.

Starch is a polysaccharide with a chemical formula of (CR 6RHR 10ROR 5R)RnR. Derived from photosynthesis, starch, which serves as the sugar reserve in plants, appears as relatively dense granules that are insoluble in cold water. The size of these granules varies from 1 to 100 µm depending on the botanical source.

III.4.1.ALPHA-AMYLASE:

Alpha-amylase is a macromolecule belonging to the class of globular proteins, specifically endoglycanases, of the hydrolase class, which acts on the α (1-4) linkages of starch. Its action initially produces a mixture of glucose, maltose, and dextrins. At the end of the reaction, it provides glucose and residues corresponding to the α (1-6) linkages located at branching points of the chains (Raimbault, 1981; Alais et al., 2008).

Alpha-amylase (EC3.2.1.1) is a digestive enzyme classified as a glycosidase. During germination, the starch in the endosperm is hydrolyzed into soluble reducing sugars under the action of alpha-amylase (Haq et al., 2002). However, if a grain is cut in half widthwise, only the half containing the embryo produces reduced sugars from starch. The embryo is necessary for the production of alpha-amylase by the aleurone layer (Haq et al., 2002). It is a plant enzyme commonly obtained by extraction from cereals, especially wheat, barley, bran, or rice (SrinivasaRao et al., 2004). It often forms during seed germination, which requires significant enzymatic activity for reserve conditioning and embryonic development (Brawn and Kelly, 1993; Charles et al., 2003).

III.4.2.ALPHA-AMYLASE ACTIVITY:

Starch reserves are maintained throughout seed dormancy, and the initiation of activity occurs during seed germination. Starch is converted into sugars that the plant utilizes for its growth. Alpha-amylase is produced during seed germination and degrades the α (1-4) linkages in amylose chains, resulting in the production of maltose (Charles et al., 2003).

Alpha-amylase is an enzyme that breaks down starch. In the seed, it is synthesized by the aleurone layer (the outermost layer of the seed) and is hormonally stimulated by the embryo.

These enzymes contribute to the complete hydrolysis of starch, which is a glucose polymer and the main carbohydrate reserve produced by plants during photosynthesis (Nielson et al., 2001; Burhan, 2003).

Alpha-amylase plays an important role in plant metabolism as it participates in the hydrolysis of starch, producing reducing sugars by breaking the α (1 → 4) glycosidic linkages within amylose and amylopectin chains, resulting in maltose and glucose molecules (α-glucose disaccharides). These molecules can be detected by the blue color of Fehling's starch solution using iodine (Lugol's iodine reagent) (Charles et al., 2003; Badot et Merlin, 1984).

III.4.3.STRUCTURE OF A-AMYLASE:

α-amylases are considered glycoproteins containing 478 amino acids distributed into two globular domains called A (1-380 residues) and B (381-478 residues). These domains are connected by a polypeptide chain consisting mainly of hydrophobic residues. The carbohydrate part is primarily composed of mannose.

The residues constituting the substrate-binding site, as well as those constituting the catalytic site, are located in domain A. This domain reveals that α-amylase is formed by eight folded β-sheets and eight α-helices (Chiba, 1988; Burhan, 2003).

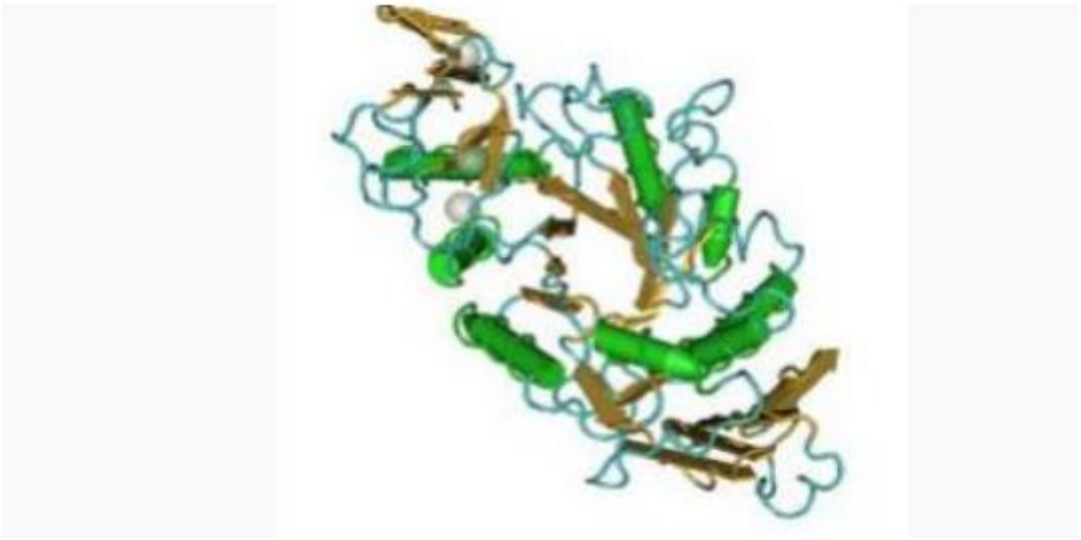


Figure n° 8: representation of the secondary and tertiary structures of alpha amylase (kadziola et al, 1994).

III.4.4.SOURCES OF A-AMYLASES:

α-amylases are enzymes produced by plants, animals, and microbes, where they play a significant role in carbohydrate metabolism (Sivaramakrishnan et al., 2006).

According to Heller et al. (2000), plant-origin α-amylase is synthesized in the aleurone layer.

Plant and microbial-origin α-amylase have been used as food additives for centuries (Sivaramakrishnanet al., 2006)

CHAPTER IV
PHYTOHORMONES

IV.1.DEFINITION OF PLANT HORMONAL SYSTEM:

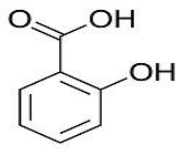
In a very general sense, a hormone is a molecule synthesized by an organism that, at very low concentrations, exerts an effect on the development of specific tissues in that organism, typically different from the ones in which the molecule was produced. This molecule carries information to the target cell and influences its functioning (Shuster, 2014).

In this regard, phytohormones or plant hormones are not strictly considered hormones (Hallé, 1999). They are highly active organic substances produced by one part of a plant body and transported to another part where, in very small quantities, they control or stimulate a different process. Moreover, they can even act as information carriers for communication between plants (Davies, 2010).

IV.2.SALICYLIC ACID:

Salicylic acid (Figure 10) or ortho-hydroxybenzoic acid belongs to a distinct group of plant phenols, which are substances with an aromatic ring to which a hydroxyl group or a functional derivative of it is attached. Plant phenols are generally classified as secondary metabolites that play an important role in regulating plant growth, development, and interactions with other organisms (Maruri-Lopez et al., 2019).

Salicylic acid, also known as 2-hydroxybenzoic acid, is a derivative of benzoic acid. It is a secondary plant product that plays a crucial role in plant growth and development. This molecule belongs to the group of phenolic compounds, which are defined as substances with an aromatic ring and a hydroxyl group or a functional derivative (Shumsul et al., 2013). It is present in various plant species to regulate biological processes such as thermogenesis, flowering, or defense against pathogens. Salicylic acid is generally present in plants in small amounts ranging from 1 to 500 μM, and its concentration increases when plants are exposed to stressful conditions, as it serves as a potent signaling molecule in plants against abiotic stress reactions (Shumsul et al., 2013).



Salicylic acid

$C_7H_6O_3$

Figure n° 9: Salicylic acid, chemical structure and formula stock illustration.

IV.3.THE ROLE OF SALICYLIC ACID IN PLANTS:

Salicylic acid (SA) is a natural phenolic compound. It plays an important role in regulating plant growth, development, maturation, and defense responses (Miura and Tada, 2014). SA plays a significant role in responding to abiotic stresses, including drought, low temperatures, and salt stress. It has been suggested that SA has great agronomic potential in improving stress tolerance in important agricultural crops (Miura and Tada, 2014).

IV.4.GIBBERELLINS DEFINITION:

During research on rice diseases caused by the fungus Gibberellafujikuroi, scientists discovered a broad group of plant growth substances known as gibberellins. As early as the 1950s, it was already known that gibberellins functioned as hormones that regulated plant growth (Brian et al., 1955). Gibberellins are involved in numerous processes related to plant growth and development, such as seed germination, leaf expansion, stem and hypocotyl elongation, floral initiation, floral organ development, fruit development, and the stimulation of specific hydrolytic enzymes in cereal aleurone (Matsuoka et al., 2003).

Gibberellins (GAs) are a diverse group of compounds sharing a common gibbane nucleus ($C_{15}H_{24}$) . There are more than 100 gibberellins designated by GAN, with “N” ranging from about 1 to 130. Gibberellin A3 is the most prevalent (Yamaguchi, 2008).

PART II

MATERIAL

AND

METHOD

MATERIAL AND METHODS:

1. PURPOSE OF WORK:

This work aims to evaluate the effect of salicylic acid and gibberellin, which could act as biostimulants, on improving the response of bean (*Phaseolus vulgaris*) seeds during the germination stage under salinity.

2. PLANT MATERIAL:

The seeds of *Phaseolus vulgaris* were provided by the company SARL AGROSEED.

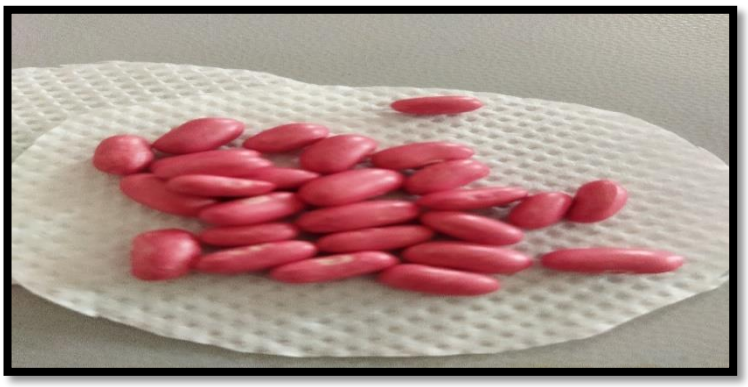


Figure n° 10: Seeds of common bean (*Phaseolusvulgaris L*) (Djaoui/Boulebene2023)

3. CONDUCT OF THE TEST:

The experimentation was conducted in the laboratory of the Faculty of Science and Technology at Tissemsilt University. The cowpea seeds were disinfected with 1% sodium hypochlorite by soaking them for 3 minutes, then rinsed with distilled water several times to remove any traces of chlorine. The seeds were placed in sterile Petri dishes with a diameter of 9 cm and a thickness of 1 cm, lined with two layers of filter paper, with 10 seeds per Petri dish. The experimental setup divided the dishes into three treatments based on the salinity level: control (0 meq), 150 meq, and 250 meq of NaCl, with 3 repetitions for each treatment. In this block, the saline solutions constituting the different germination media were prepared using distilled water. Three other similar setups were included in the study: the first involved the exogenous application of 0.5 mM salicylic acid, a signaling molecule, to counteract the harmful effects of salinity. The second involved the application of gibberellin at 150 meq and 250 meq. In each Petri dish, the germinating seeds were soaked in 20 ml of the salinesolution. During the germination tests, the Petri dishes were maintained at a temperature of 26 °C.

		
Put the seed in sterile Petri dishes	Put the Petri dishes inside the oven	Temperature setting at26 °C.

Figure n° 11: Prepare Petri dishes and place them inside the oven (Djaoui/Boulebene2023)

4. PARAMETERS MEASURED:

4.1THE FINAL GERMINATION RATE:

This rate is obtained by the number of seeds germinated from the beginning to the end of the germination.

5. BEAN SEED AMYLASE ACTIVITY:

5.1. IMBIBITIONS TEST:

The second part is based beforehand on the evaluation of the imbibition of the seeds germinated in different germination media, by weighing the seeds every day, until the appearance of the radicle (start of growth). This test is necessary to determine the appropriate time for the extraction and analysis of amylases.

5.2. ASSAY OF AMYLASE ACTIVITY OF GERMINATING SEEDS:

During seed germination, amylase activity is essential for the remobilization of carbohydrate resources, stored in the form of starch.

The amylase activity is measured according to the method of Bernfeld (1955). The principle of this method is based on measuring the reducing power of maltose released during the enzymatic hydrolysis of starch. The color intensity is proportional to the amount of maltose released (Bernfeld, 1955). The enzymatic activity is expressed per µmole of maltose released per minute

5.3. EXTRACTION OF THE ENZYME COMPLEX:

The extraction of the enzymatic complex is produced in two stages of the seed germination process, 24h. The extraction substrate consists of 4 g of seeds from different germination media. The whole is ground in 12 ml of acetate buffer solution at pH 4.8, and filtered. The filtrate is collected in a 1.5 ml eppendorf tube, then centrifuged for 10 min at 8000 g. The supernatant is recovered (extractA).

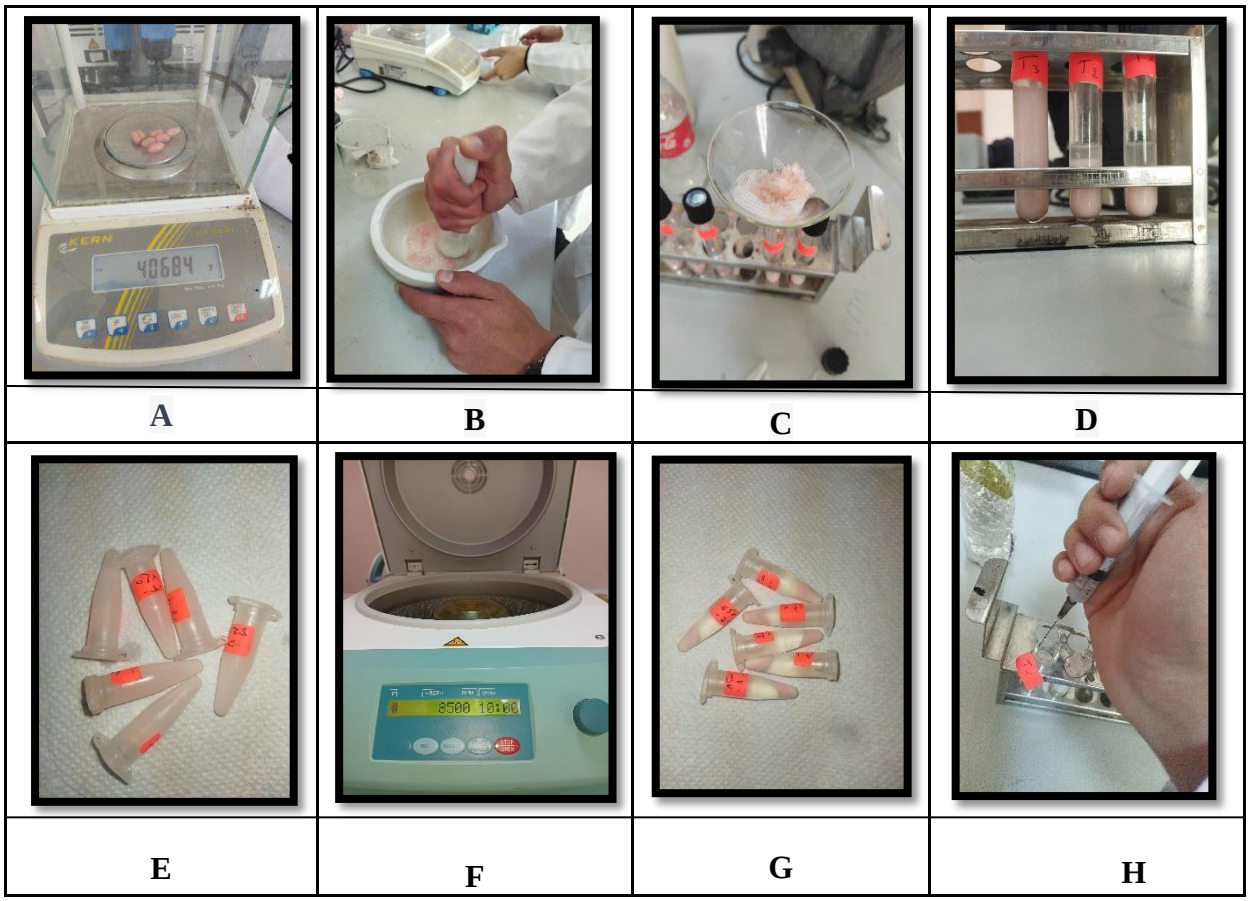


Figure n° 12: Steps to extract the enzyme complex (Djaoui/Boulebene2023)

5.4. DETERMINATION OF AMYLASE ACTIVITY:

In test tubes, add 1 ml of the extract (A) to 0.5 ml of a 1% starch solution prepared with starch and acetate buffer at pH 4.8. Vortex the mixture and incubate it in a water bath at 25°C for 10 minutes. Add 0.5 ml of the reagent (A) containing 28 ml of 2N NaOH, 0.4 g of dinitrosalicylic acid (DNS), 12 g of tartrate (K/Na), and 40 ml of distilled water. This reagent inhibits hydrolysis and allows for simultaneous measurement of the formed maltose. Place the mixture in a water bath at 100°C for 5 minutes, and then let it cool. The optical density was measured using a spectrophotometer at a wavelength of λ=530 nm.

Figure n° 13: test tubes after putting them in the water path.(Djaoui/Boulebene2023)

6. STATISTICAL ANALYSIS:

Statistical analysis of the data was performed using 2-way and multi-way analysis of variance (ANOVA), using OriginiPro2022)

RESULTS AND DISCUSSION

I.INTERPRETATION OF RESULT:

1.GERMINATION RATE:

Table I: Analysis of variance of germination rate as a function of salt stress and biostimulants.

	F Value	P Value
Saltestrees	196,77273	5,85723E-13
Extrai	8,59091	0,01569
Interaction	4,09091	5,69574E-11

At the 0.05 level, the population means of **Salt stress** are **significantly** different.
At the 0.05 level, the population means of **Extrai** are **significantly** different.
At the 0.05 level, the interaction between **Salt stress** and **Extrai** is **significant**.

The analysis of variance (Table I) indicated that the germination rate of bean seeds is significantly affected by the variation in salt concentration, the added extracts, and their interaction ($p < 0.05$).

The obtained results (Figur 14) demonstrated that salinity greatly decreased the germination rate, reaching values of 23% at 150 meq and 10% at 250 meq. However, this rate averaged around 100% at 0 meq.Gibberellin and salicylic acid significantly improved this parameter with increasing salinity levels. In fact, the germination rate reached approximately 100%, 60%, and 23% for gibberellin, and 100%, 56.7%, and 16.7% for salicylic acid at 0 meq, 15 meq, and 250 meq, respectively.

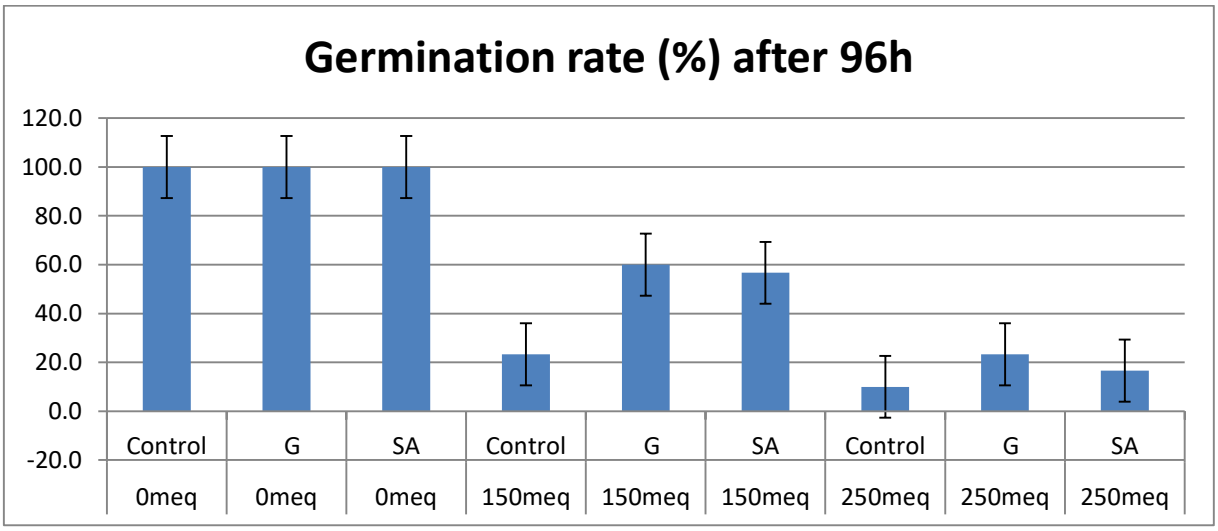


Figure n° 14: Germination rate (%)as a function of salt stress and biostimulants

2. AMYLASIC ACTIVITY (MG/G.FM/T)

Table II: Analysis of variance of Amylasic activity as a function of salt stress and biostimulants.

	F value	P value
Salte stress	3,20002	0,0647
Extrai	27,44716	3,41357E-6
Interaction	4,25269	0,01348

At the 0.05 level, the population means of **Salt stress** are **not significantly** different.
At the 0.05 level, the population means of **Extrai** are **significantly** different.
At the 0.05 level, the interaction between **Salt stress** and **Extrai** is **significant**.

The analysis of variance (Table II) highlighed that the amylasic activity of bean seeds is not depend on salt stress ($p \geq 0.05$). However this parameter is significantly affected by the added extracts and their interaction with salin level ($p < 0.05$).

According to the results (Figure 15), it has been revealed that salinity slightly decreased the amylase activity. In fact, the recorded values were 0.1, 0.1, and 0 (mg/g.MF/t) at 0 meq, 150 meq, and 250 meq, respectively. However, Gibberellin and salicylic acid significantly improved this activity with increasing salinity levels. In fact, the amylase activity reached approximately 0.2 (mg/g.MF/t) for gibberellin at all salt levels. Additionally, these values were 0.2, 0.2, and 0.3 (mg/g.MF/t) for salicylic acid at 0 meq, 150meq, and 250 meq, respectively.

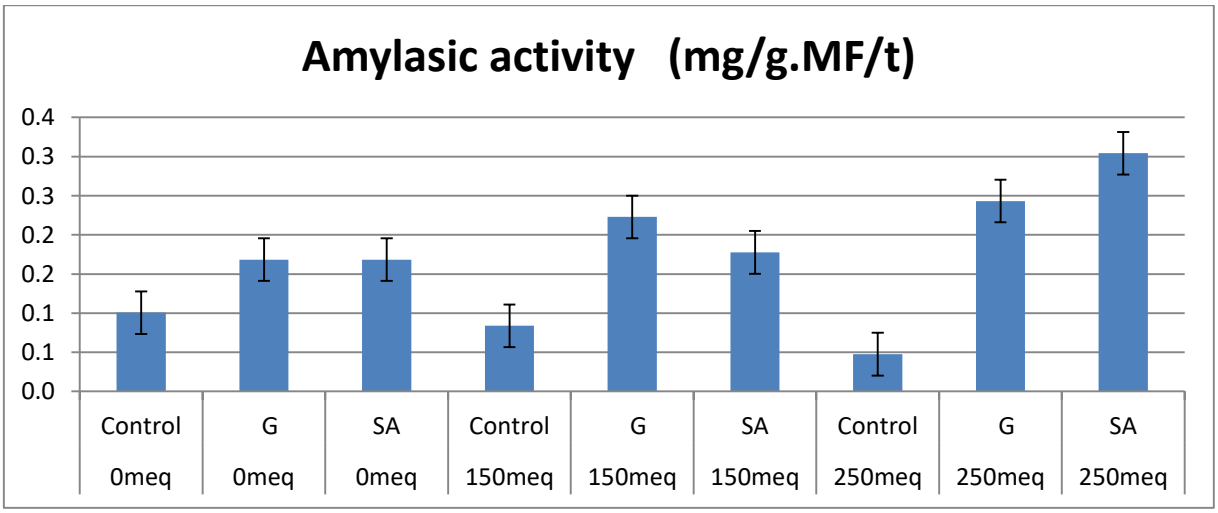


Figure n° 15: Amylasic activity (mg/g.MF/t)as a function of salt stress and biostimulants

3. DISCUSSION:

According to RHS (2021), germination is the process through which a seed initiates growth and development, involving the breaking of dormancy and the emergence of a radicle and shoot. However, germination can be impeded by various abiotic stresses, such as salt stress, which negatively impacts seed germination by affecting cellular membrane integrity, enzymatic activity, and inducing oxidative stress (Gao et al., 2019).

Our study specifically focused on common bean (*Phaseolus vulgaris*) and demonstrated that salinity reduced the final germination rate at concentrations of both 150 and 250 meq/l. Salinity is a significant stress factor responsible for inhibiting germination percentage and delaying crop germination time (Zörb et al., 2019; Shahet et al., 2020). Hence, salt stress disrupts the water status of plants (Kamran et al., 2020; Sofy et al., 2020). Nonetheless, the application of phytohormones, including gibberellin and salicylic acid, has shown promising results in improving the germination rate of common bean (Phaseolus vulgaris).Camara et al. (2018) have confirmed that Gibberellin plays a role in seed germination, abiotic stress responses, improved fruit growth, stem elongation, flowering, and other physiological effects when interacting with other plant hormones.

AccordingtoMaruri-Lopez et al. (2019), salicylic acid serves as a regulator of plant growth, development, and interaction with other organisms. Salicylic acid (SA) is a natural phenolic compound that plays a crucial role in regulating plant growth, development, maturation, and defense responses (Miura and Tada, 2014). SA also plays a significant role in responding to abiotic stress, including drought, low temperatures, and salinity. It has been suggested that SA exhibits great agronomic potential for enhancing stress tolerance in important agricultural crops (Miura and Tada, 2014).

During seed germination, amylase activity plays a vital role in mobilizing carbohydrate reserves stored as starch. Analysis of the results indicates that the activity of amylases extracted from germinating seeds is not influenced by different saline treatments.

Furthermore, the exogenous contribution of gibberellin does not affect amylase activity. Therefore, it can be inferred that salinity affects amylase functioning, but not its synthesis, as demonstrated in a study by Kaplan et al. (2004). Maltose, which accumulates due to β-amylase induction, has been shown to protect proteins, membranes, and the photosynthetic electron transport chain under acute temperature stress.

CONCLUSION

Conclusion:

Germination is a process involving biochemical changes in grains throughout its progression. It is considered a crucial stage for establishing subsequent stages of crop growth and ensuring their successful development. However, this phase is greatly affected by abiotic stresses, particularly salinity. Two parameters were studied in our research, namely the germination rate estimated at 96 hours and the amylase activity estimated at 24 hours. The increase in salinity level strongly influenced both of these parameters. Conversely, the addition of gibberellin and salicylic acid, which was another objective of this study, improved the values of these two parameters while promoting the germination of common bean grains. Finally, it is important to study the effect of this stress at this vegetative stage in order to ensure proper establishment of the crop and enhance our understanding of resistance and sensitivity mechanisms. Gibberellin and salicylic acid are considered biostimulants that have garnered increasing interest in recent years.

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Figure n° 01: Description of the Bean plant
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figure n°02 : Common bean flower"Phaseolus vulgaris" by Dingilingi
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Figure n°03 : Haricot fruit Dessin Chantal Beaumont - Archives Larousse
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Figure n°4: Phaseolusvulgariscropdevelopment cycle DIAW NF (2002) Utilisation des inoculums de rhizobium pour la culture du haricot(Phaseolusvularis) au Sénégal. Thèsedoctorat.UniversitéCheikh Anta Diop. Dakar,97 p.

Figure n° 5: Deleterious effects related to salinity stress toxicity and the cellular response set up to ensure tolerance. In halophilic plants, excess salt in the soil causes ionic, oxidative and osmotic stress, which they must manage by implementing strategies to maintain (A) oxidative stress molecules at an acceptable level, (B) osmotic balance, and (C) ionic homeostasis. [Source: © EEnv diagram]

Figure n° 6: Schematic representation of Phaseolus vulgaris L. plant, 8 weeks old, showing vegetative and reproductive structures source: https://scialert.net/fulltext/?doi=ijb.2010.323.333)

Figure n°7: Influence of environmental conditions on seed development, dormancy and germination. N'Dri. A.A.N., Vroh-Bi I., Kouamé. P.L., Zoro Bi. I.A.∗(2011). Bases génétiques et biochimiques de la capacité germinative des graines : implications pour les systèmes semenciers et la production alimentaire. Sciences & Nature, 8: 119-137.

Figure n°08: representation of the secondary and tertiary structures of alpha amylase. Kadziola , A., Abe , J., Sevensson , B. and Haser, R .1994. Crystal and molecular structure of barely alpha-amylase. Journal of molecular structure biology.293(1),104-21.

Figuren°09: Salicylic acid, chemical structure and formula stock illustration https://www.istockphoto.com/vector/salicylic-acid-chemical-structure-and-formula-gm1270238713-373255803

Figure n°10: Seeds of common bean (*Phaseolus vulgaris L*) (original picture)

Figure n°11: Prepare Petri dishes and place them inside the oven (original picture)

Figure n° 12.:Steps to extract the enzyme complex (original picture)

Figure n° 13: test tubes after putting them in the water path. (original picture).

Figuren° 14: Germination rate (%)as a function of salt stress and biostimulants

Figure n° 15:Amylasic activity (mg/g.MF/t)as a function of salt stress and biostimulants

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