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**MINISTRY OF HIGER EDUCATION AND SCIENTIFIC RESEARCH**

**N$^0$ Réf : ……**


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**Institute of Science and Technology**

**Department of Life and Natural Sciences**

**Field: Life and Natural Sciences**

**Option: Plant biotechnology**

**Speciality: Plant biotechnology**


***Theme:***

# Salinity effect on germination and Proline synthesis of the leguminous species: *Vigna uniguiculata (L.) Walp., 1843*


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# Acknowledgment

***In the Name of ALLAH, the Most Gracious, the Most Merciful***
***Before all, none of this would have been achieved without ALLAH's support and guidance.***
***For most praise be to Our Lord, ALLAH***

***We are deeply indebted to our teacher Dr. TORCHE Yacine for his valuable support, direction, and time he devoted to crafting our work throughout the writing of this letter. Without his thoughtful comments and encouragement, this work would not have seen the light.***

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***We would like to extend our special thanks to all the teachers who enlightened our path throughout our university studies***

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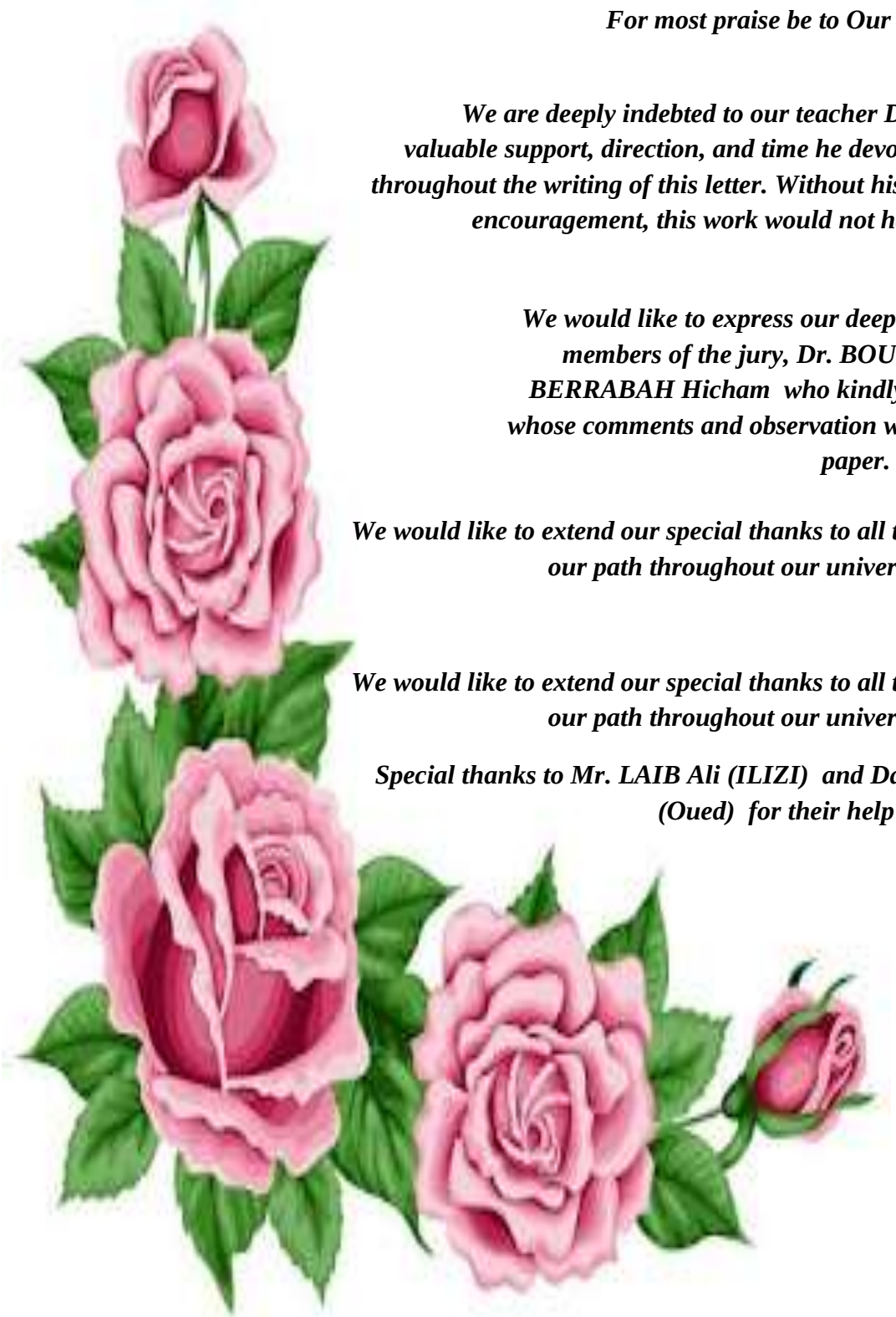

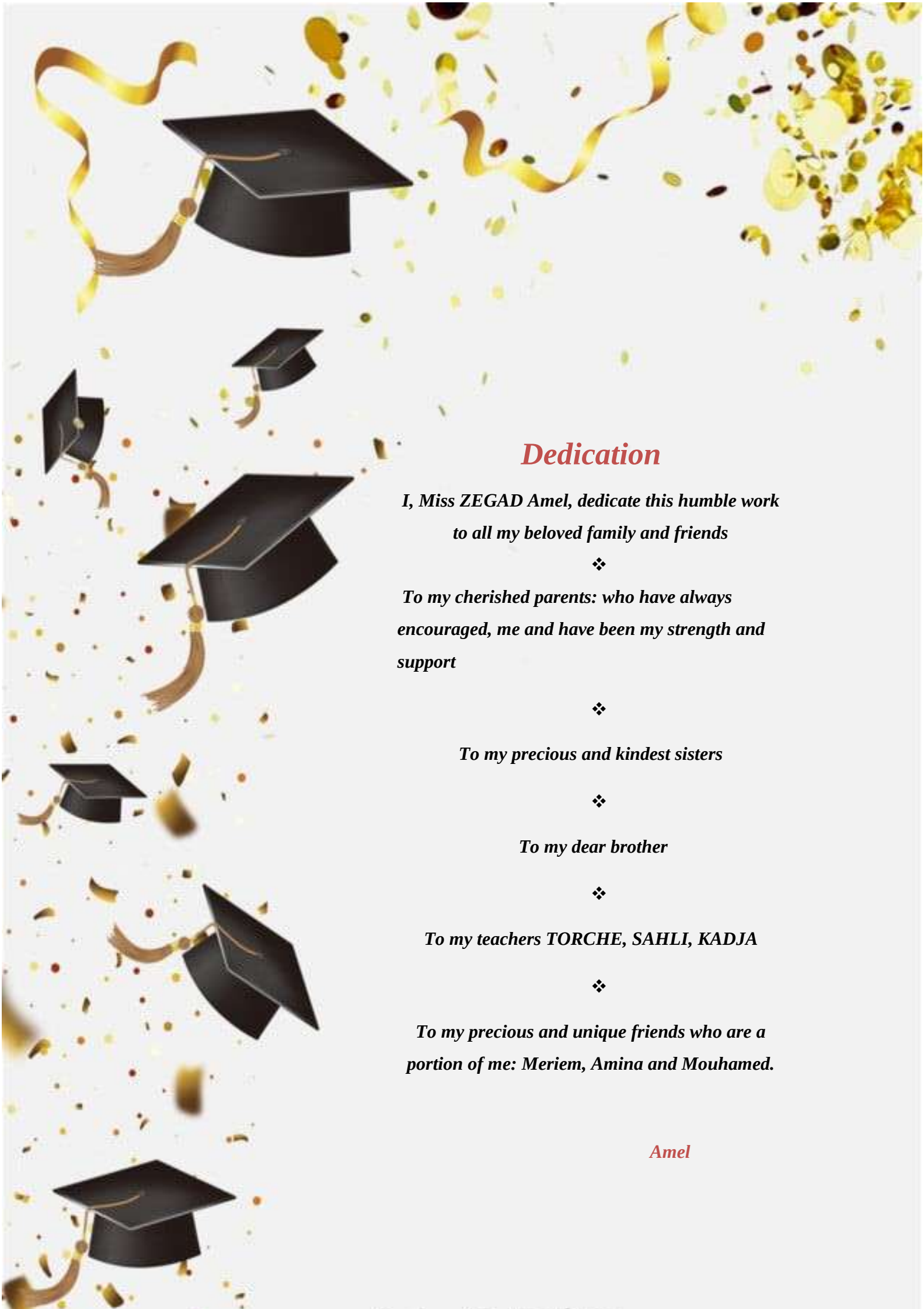

# *Dedication*

***I, Miss ZEGAD Amel, dedicate this humble work to all my beloved family and friends***

❖

***To my cherished parents: who have always encouraged, me and have been my strength and support***

❖

***To my precious and kindest sisters***

❖

***To my dear brother***

❖

***To my teachers TORCHE, SAHLI, KADJA***

❖

***To my precious and unique friends who are a portion of me: Meriem, Amina and Mouhamed.***

***Amel***

# *Dedication*

***In the Name of Allah, I would like to dedicate this humble work to:***

***The dearest people to my heart, to the sun that lightens my life, my sweetheart, my dear mother Madejda and my kind father Mosbah may ALLAH bless them***

❖

***To my brothers: Mohamed and Amir***

***Thank you for your precious and priceless support.***

❖

***To my sisters: Chirine and Nermine***

***Thank you for being there when I needed you and supporting me with all that you have***

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***To my flowers: my partner and my beloved friend, Rima, Zineb, Imane and Asma***

***To all my dear cousins***

***Thank you for the nice times that we spent together***

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***To my teacher TORCHE Yacine and my sweet heart BEDJAOUI Mira who I will never forget***
***To everyone who helped me one day***

***Nourhane***

# *Résumé*

Le présent travail a pour but d'étudier l'effet du stress salin au stade de germination et de stade de plantule de 11 variétés de niébé (*Vigna unguiculata subsp. unguiculata (L) Walp),* cultivées et commercialisées en Algérie. L'étude a été réalisée dans le laboratoire des sciences et des matières du centre universitaire d'Abd Al Hafid Bousouf de Mila, dans des condition favorable au culture (humidité , lumière, température 25 C°) Les graines ont été mises à germer dans des boites de Pétri contenant des concentrations croissantes de NaCl (0mmol/l, 50mmol/l, 100mmol/l, 150mmol/l). Les paramètres de germination ont été évaluer notamment (Vigueur d'index, Cinétique, Taux de germination, La précocité de germination, la teneur de proline). Dans une seconde partie pour la détermination de la proline des graines ont été mises en pot de 15/10 cm remplis avec de la tourbe noir jusqu'au stade plantule, les traitements salin ont étaient appliquées de la même façon pour la germination. Les résultats démontrent que le sel a un effet négatif sur le taux de germination, cependant elle augment le taux de proline dans les génotypes (O) et (I).L'effet de salinité varie en fonction de la variété et de l'intensité du stress.

**Mots clés :** Niébé, Stress salin, Germination, Na Cl, Proline.

# *Abstract*

The aim of this work is to study the effect of salt stress at the germination and seedling stage of 11 varieties of cowpea (*Vigna unguiculata subsp. unguiculata* (L) Walp), cultivated and marketed in Algeria. The study was carried out in the science and materials laboratory of the university center of Abd Al Hafid Boussouf of Mila, in a favorable growing condition (ambient humidity, light, temperature 25 C° (The seeds were germinated. In Petri dishes containing increasing concentrations of NaCl (0mmol/l, 50mmol/l, 100mmol/l, and 150mmol/l). The germination constants were calculated (Index vigor, Kinetics, Germination rate, early germination, the proline content). In a second part for the determination of the proline the seeds were placed in a 15/10 cm pot with black peat until the seedling stage, the salt treatments were applied with same way for germination. The results show that salt has a negative effect on the germination rate and the increase of proline levels in some genotypes (O) and (I). However, this effect varies according to the variety and the intensity of the seed stress.

**Key words:** Cowpea, Salt stress, Germination, NaCl, Proline.

# الملخص

الهدف من هذا العمل هو دراسة تأثير الإجهاد الملحي في مرحلة الإنبات والشتلات لـ 11 نوعًا من الفاصولياء (*Vigna unguiculata subsp. unguiculata* (L) Walp)، المزروعة والمُسوَّقة في الجزائر. أجريت الدراسة في مخبر العلوم والمواد بالمركز الجامعي عبد الحفيظ بوالصوف، ميلة، في ظروف نمو ملائمة (رطوبة، ضوء، درجة حرارة 25 درجة مئوية)، حيث أنتشت البذور في علب بتري تحتوي على تراكيز متزايدة من كلوريد الصوديوم (0 ملي مول / لتر، 50 ملي مول / لتر، 100 ملي مول / لتر، 150 ملي مول / لتر) تم حساب ثوابت الإنبات (قوة المؤشر، الخواص الحركية، معدل الإنبات، سرعة الإنبات، محتوى البرولين). وضعت البذور في وعاء 10/15 سم مع المادة العضوية حتى مرحلة الشتلات، وطبقت معاملات الملح بنفس طريقة الإنبات، وأظهرت النتائج أن للملوحة تأثير سلبي على معدل الإنتاش في حين انها ساهمت في زيادة نسبة البرولين. في بعض الأنماط الجينية (o) و(I)، إن تأثير الملوحة يختلف باختلاف التنوع الجيني وشدة إجهاد البذرة.

**الكلمات المفتاحية:** فاصوليا العين السوداء، الإجهاد الملحي، الإنتاش، كلوريد الصوديوم، البرولين.

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# *List of abbreviations*

- v. u *: vigna uniguiculata*
- var : varieties
- cm : centimetre
- mm : mili metre
- m : metre
- MMT :million metric tonnes
- TT : Noir Tadleghat
- E : Vigna Arabia
- I : Ilizi
- O : Oued
- TZ : Vigna Tizi Ouzou
- AZ : Vigna Azazga.
- RA : Red Aoulef.
- NA1 : Niébié d'Asie.
- NA2 : Niébié d'Australie
- NB : Niébié Brezil.
- NE : Niébié Egypt.
- ABA: Acid ABscissic.
- $Ca^{2+}$ : Calcuim
- NaCl: Sodium Chloride.
- SOS :Salt Overly sensitive
- NHX; Vacuolar Na + / H + exchanger
- HTK1; Histidine kinase.
- DNA: Desoxyribos kuclic Acid.
- $K^{+}$: Potassuim.
- APX: Ascorbate Peroxidise.

- CAT: Catalase.
- PCD: Programmed cell death.
- ROS : Reactive Oxygen Species.
- mmol : mili Mole.
- i. e : It Is Means.
- S0: control condition.
- S1:50 mmol/l.
- S2:100 mmol/l.
- S3 : 150 mmol/l

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# *Introduction*

## Introduction

Cowpea is a grain legume belonging to the order of legumes, the family *Fabaceae*, the tribe *Phaseolea*e and the genus Vigna (**Maréchal and al, 1978),** the species Vigna *unguiculata* is one of the main food legumes cultivated in different parts of the world **(West and Francois, 1982; Kouadio and al, 2007)**, it faces numerous production constraints, including abiotic stress, in particular salinity, which seriously affects its productivity (**Lawlor, 2013).**

In Algeria, cowpea (*Vigna unguiculata*) is commonly called, Lubia kabyle en Kabylie, Tadelaght in the southern oases or Lubia arebi in the region of El Kala. It is traditionally cultivated and consumed in certain regions such as Kabylie, the area east of the wilaya of El Taref and the oases of the Sahara (**Ghalmi and al, 2005).**

Salinization is a major ecological problem that affects a number-growing region of the globe, frequently associated with water stress; it reduces arable land and threatens the global food balance (**Derkaoui, 2011).**

The semi-arid, arid zones constitute about two-thirds of the surface of the terrestrial globe (**Ben Brahim and al, 2004)**. In these areas often marked by severe periods of drought, soil salinization is considered one of the main factors limiting the development of plants.

Globally, nearly 400 million hectares are affected by phenomenon of desertification (**Jouve and al, 2002),** of which 10 million are considered affected by salinity (**Munn, 2002).** Agricultural areas affected by salinity in the world would be 340 million ha or 23% of the land cultivated (**Cheverry, 1995**), including 3. 2million hectares of land threatened by salinity in Algeria (**Belkhodja and al, 2004).**

According to **Murillo and al, (2001),** Salinity delays germination of cowpea seeds and reduces the percentage of germination. In stressed plant species has been correlated with the ability of plant to accumulate the proline and its concentration is generally higher in tolerant plant than sensitive plant (**Hanana and al, 2011**).

In view of the spread of salinity in Algeria, which causes major problems, especially in the agricultural aspect, which is an important element in its economy, we discussed conducting this study at the University Center Abdel El Hafid Boussouf, which aims to study the effect of salinity on the germination and biosynthetic of proline in cowpea (*Vigna Unguiculata)*, which is known in some countries for its resistance to salinity. Where, this work divides into three chapters.

The first is the bibliographic chapter which contain two parts, the first part presents a bibliographic study on cowpea, while the second part contain the salinity and its effect on plant and the mechanism of plant to tolerate this problem.

The second chapter is present the materials and methods of this work we will write all the steps in detail to study the effect of salinity on cowpea germination however the second part presents the results obtained and their discussion.

# *Chapter I:*

# *Bibliography*

# *Cowpea (vigna unguiculata (L.) Walp 1984)*

# I. Cowpea (*Vigna Unguiculata (L.) Walp)*:

## 1. History, Origin and Distribution:

Cowpea (*Vigna unguiculata*) is one of the oldest human food sources and has probably been used as a crop plant since Neolithic times (**Summerfield and al, 1974).**

The lack of archaeological proves has resulted in contradicting views supporting Africa, Asia, and South America as origin for cowpea (**Kaplin and Lynch, 1999).** One view is that cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago (**Allen, 1983).**

Before 300 BC, cowpea had reached Europe and possibly North Africa from Asia. In the 17th century AD, early in the 18th century the Spanish took the crop to West India. The slave trade from West Africa resulted in the crop reaching the southern USA (**Padulosi and Ng, 1997).**

Another view was that due to the presence of most primitive wild varieties the Transvaal region of the Republic of South Africa was the centre of *V. Unguiculata (***Padulosi and Ng, 1997).** Presently, cowpea is growing throughout the tropic and sub tropic areas of the world.

## 2. Species or Taxonomic group:

## 2.1. Classification and Nomenclature:

Cowpea (*Vigna unguiculata (*L.) Walp.) Belongs to the family *Fabaceae*genus *Vigna*, and section *Catiang (***Verdcourt, 1970; Maréchal and al, 1978)**.

**Kingdom:** Plantae

**Division:** Magnoliophyta

**Class:** Magnoliopsida

**Order:** Fabales

**Family:** Fabaceae

**Sub-family:** Faboideae

**Tribe:** Phaseoleae

**Sub-tribe:** Phaseolinae

**Genus:** Vigna

**Species:** unguiculata

Annual cowpea has two botanical varieties, the cultivated *Vigna unguiculata unguiculata* var. *unguiculata* and the wild form *V. u. u.* var. *spontanea*, both of which are inbreeding. *V. u. u.*, var. *spontanea* is typically found mostly near the borders of cultivated cowpea fields and within them. Cultivated cowpeas have been divided into five cultivar groups (Table 1) based mainly on pod, seed and ovule characteristics (**Pasquet, 1999)**.

*Unguiculata* is the largest cultivar group. The cultivar group Sesquipedalis (variously known as "asparagus bean", "yardlong bean", "long bean" or "snake bean") has more than 16 ovules and seeds spaced within the pod. Recent molecular evidence suggested that it is a subspecies (**Xu and al, 2012).**

**Table 1:** The five-cultivar groups of cowpea.

| Cultivar group | Selected feature |
|---|---|
| **Unguiculata** | Includes most African grain and forage types. More than 16 ovules/pod. |
| **Biflora (Catiang)** | Smooth seed in short erect pods. Common in India. Less than 17 ovules/pod. |
| ***Melanophthalmus*** | Blackeye pea types. Less than 17 ovules/pod. Grown mostly in the Americas |
| ***Sesquipedalis*** | Asparagus or yard-long beans. Very long pods consumed fresh, especially in the People's Republic of China |
| **Textilis** | Rare form with very long peduncles once used for fibre in Africa. |

### 2.1.1. Scientific Name: *Vigna Unguiculata*

Common names: in arabe (Arabic), Niébé (French), Cowpea (English), Koso (Moru), Loputu (Bari), Akuem (Dinka), Ngor (Acholi), Amodoro (Latuka), Osu (Madi), Omodoro (Lokoya), Moro (Dongotono), Anyege (Zande), Namodoro (Lango), Okia (Nuba), and Ngor yamg (Nuer) (**Tony, 2015).**

The wild cowpeas in the subspecies *unguiculata* currently are described as being the variety *spontanea (***Padulosi. 1993).** Var. *spontanea* are similar to domesticated cowpea landraces except that the pods are small and dehiscent, and the seeds are ten times smaller than cultivated cowpea. The seed coat of *spontanea* is hard, thick and impermeable to water. There are no obvious barriers to hybridization or recombination between members of these five different cultivar groups or with the wild cowpeas (var. *spontanea*) in the subspecies *unguiculata*.

The *Vigna unguiculata* species complex is currently divided into 11 subspecies (**Padulosi, 1993; Padulosi and Ng, 1997; Pasquet, 1997, 1993a, 1993b**).

## 3. Description of the plant:

The cowpea (*Vigna unguiculata (*L) Walp), is an annual herbaceous legume cultivated for its edible seeds or for fodder. Cultivated cowpeas are herbaceous annuals that are either, or climbing annuals with a taproot and all are glabrous. They are mostly grown for grain but a small proportion (about 10%) are grown as fresh pods in eastern Asia or as green leafy vegetables and fodder in Africa (**Boukar and al, 2015).**

Cowpea *V. unguiculata* can grow from 80 cm to 2 m for climbing cultivars. It has a well-developed root system. Germination is epigeal with the first pair of true leaves being simple and opposite and subsequent leaves being trifoliate with oval leaflets (6-15 cm long and 4-11 cm broad) and alternate. The *papillonaceous* flowers are racemose inflorescences at the ends of peduncles this arise from leaf axils and can be white, yellowish, pale blue or violet. Peduncles are stout, grooved, and usually much longer than the leaves (2-20 cm long). For each inflorescence, flowers are sequentially produced in alternating pairs on thickened nodes at the tip with cushion-like extra-floral nectaries between each pair of flowers. The flower is large (standard is 2-3 cm in diameter), with a straight keel, diadelphous stamens (one free and nine fused), a sessile ovary with many ovules, and a style that is bearded along the inside and ends in an oblique stigma. Pods occur in pairs forming a V, mostly pending and vertical, but they can. They are cylindrical, 2-6 cm long and 3-12 mm broad and contain 8-20 seeds. Seeds can be white, pink brown or black (**Heuzé and al, 2013)**.


**Figure 1:** Aerial parts of cowpea (*Vigna unguiculata (L.) Walp),* (**Steward, 1958)** (1).

The corolla is yellowish-white to violets-white with violet wings and mature seed colours vary from white through brown to black.

**Figure 2 :** Cultivated cowpea flower, pods and seeds (2).

(**OECD, 2018)**

**Note (1)***:* This line drawing shows leaves, stems, petioles, flowers and pods (main image), together with their productive organs consisting of stamens (nine fused and one free) and pistil with its curved style with brush below the stigma (bottom left) and parts of the corolla (bottom right); the standard (top), two wings (middle) and keel (bottom).

**Note (2**)*:* Picture of cowpea flower (top left), immature green pod (top right), maturing pods with an illustration of the great variety of seed colours (bottom).

## 4. Geographic distribution, habitats, crop production, centres of origin and diversity:

### 4.1. Geographic distribution:

Cultivated cowpeas are growing as warm-season-adapted annuals in tropical and subtropical zones as defined by **Hall (2001**).

In all countries in sub-Saharan Africa and in Asia, South America, Central America, the Caribbean, the United States and around the Mediterranean Sea. In subtropical zones, temperatures are only suitable for cowpea in the summer, whereas temperatures are suitable year-round in tropical zones. The vast majority of the world's cowpea production (over 95%) takes place in sub-Saharan Africa, with about 12. 5 million hectares under cultivation worldwide in 2014 (**Singh and al, 2002; FAOSTAT, 2014)**.

Asia is the second largest producing region, representing less than 3% of the global production in average over the 1993-2014 period, most of it being cropped in Myanmar (**FAOSTAT, 2014).**

In Africa, cowpea can be cultivated up to 1 800 m altitude but is mainly grown in the lowlands. The centre of maximum diversity of cultivated cowpeas and land races is found in West Africa in a region comprising the Sudan savannah zone of Nigeria (at 4 million ha, Nigeria has the largest area of cowpea cultivation according to **FAO STAT, (2014)**.

**Table 2 :** Global production of cowpeas (dry) in million metric tonnes (MMT): **FAOSTAT (2014)**.

| Cowpea production | Average 1993-2014 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|
| **World** | 4. 59 | 6. 91 | 4. 78 | 8. 25 | 8. 03 | 5. 59 |
| **Africa** | 4. 37 | 6. 57 | 4. 50 | 7. 95 | 7. 78 | 5. 35 |
| **Including -Nigeria** | 2. 53 | 3. 37 | 1. 64 | 5. 15 | 4. 63 | 2. 14 |
| **Niger** | 0. 79 | 1. 77 | 1. 52 | 1. 33 | 1. 63 | 1. 59 |
| **Burkina Faso** | 0. 37 | 0. 63 | 0. 44 | 0. 60 | 0. 58 | 0. 57 |
| **Tanzanie** | 0. 13 | 0. 15 | 0. 17 | 0. 18 | 0. 19 | 0. 19 |
| **Cameron** | 0. 10 | 0. 15 | 0. 16 | 0. 15 | 0. 17 | 0. 17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Mali** | 0. 10 | 0. 13 | 0. 13 | 0. 14 | 0. 17 | 0. 15 |
| **Kenya** | 0. 07 | 0. 07 | 0. 08 | 0. 11 | 0. 13 | 0. 14 |
| **Asia** | 0. 13 | 0. 23 | 0. 19 | 0. 19 | 0. 15 | 0. 15 |
| **Myanmar** | 0. 11 | 0. 21 | 0. 17 | 0. 16 | 0. 12 | 0. 12 |
| **American** | 0. 06 | 0. 09 | 0. 07 | 0. 09 | 0. 08 | 0. 09 |
| **Europe** | 0. 03 | 0. 03 | 0. 03 | . 0. 02 | 0. 02 | 0. 02 |
| **Oceania** | 0. 00 | 0. 00 | 0. 00 | 0. 00 | 0. 00 | 0. 00 |

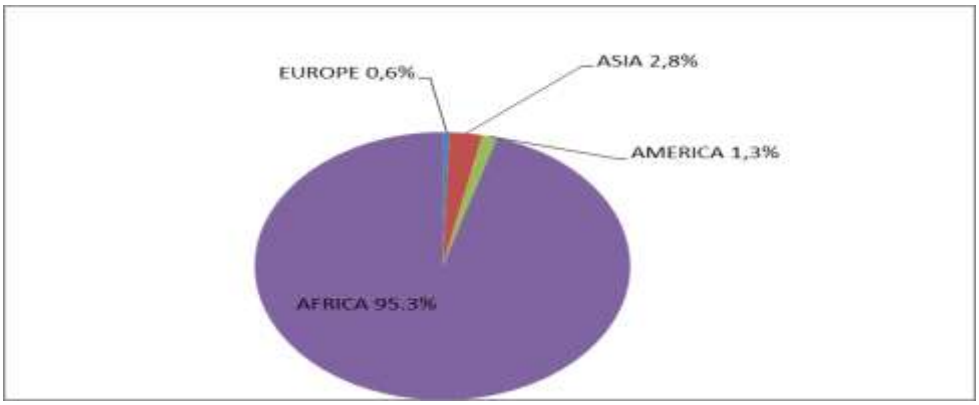



**Figure 3 :** Cowpea production share by region, (**FAO STAT, 2014).**

The wild species of cowpea are widely distributed across sub-Saharan Africa (Figure 4). They occupy a range of habitats to an elevation of 2 600 m. *Vigna monantha* has been found in Somalia in the coastal plain from Hobyo to Bender Bayla.


**Figure 4 :** Distribution of the wild species of cowpea in Africa (**Pasquet, 1996).**

## 4.2. Other regions of the world:

In Asia, both sole cropping and intercropping are practiced and large growers and in Brazil have adopted similar modern farming practices to produce high yields but in India, some intercropping of cowpea are still practised, but the majority of the crop is produced under sole cropping with inputs (**Pandey and Ngarm, 1985).** While in the United States generally only sole-crops are grown and the production is entirely mechanized with machinery and agronomic practices adapted from other crops (**Higgins and al, 2012)** but in China usually intercropped with common bean or cucumber, as pod quality/appearance, rather than yield (**Freire and al, 2011).**

The United State is the most substantial producer and exporter of cowpea but in Australia cowpea grown as a green manure cropping coastl surgical areas as a forage or dual-purpose grain ⁄ forage (**Higginset and al, 2012).**

In China *asparagus* bean is usually intercropped with common bean or cucumber, the immature pods of *asparagus* bean remain the dominant production, as pods quality ⁄ appearance rather than yield (**Freire and al, 2011).**

In Brazil and Asie, both sole cropping and intercropping are practiced (**Pandey and Ngarm, 1985).**

## 4.3. In Algeria:

The sites of Cowpea traditional cultivation in Algeria have been identified and located throughout the country.67 sites were prospected among them there is Tizi Ouzou and Kala, and in the Sahara find in Becher, Adrar and Djanet (**Tellah, 2016).**

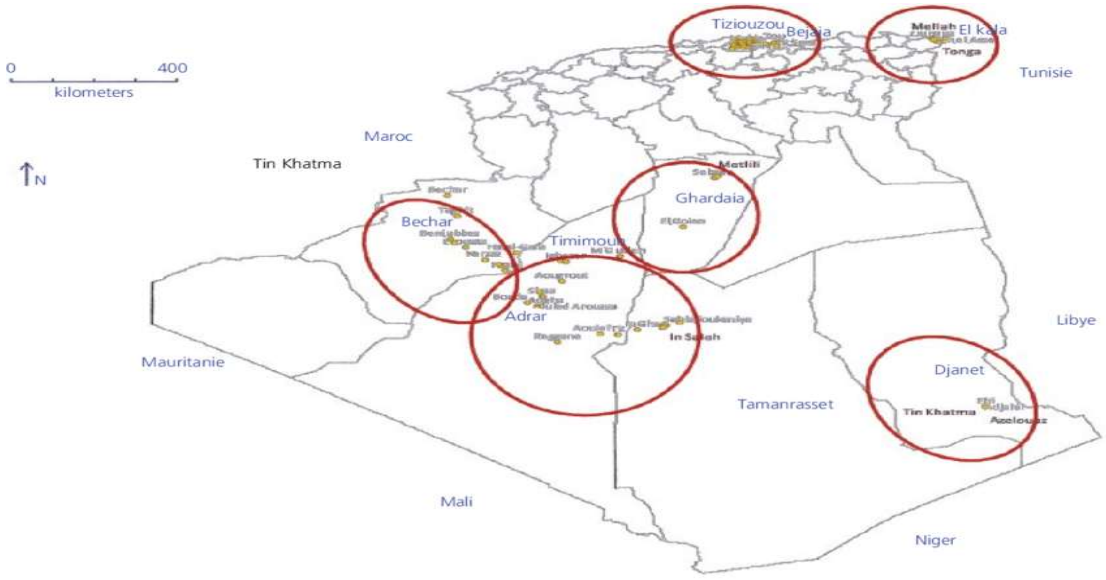



**Figure 5**: Traditional areas of the culture of cowpea in Algeria (**Tellah, 2016).**

## 5. The importance of cowpea:

Cowpea is mainly consumed as dry grain or fresh vegetable. The grain contains high protein (23% to 25%), carbohydrate, vitamins, and fibre (**Alemu, 2015).** In addition, it has low fat content, which is important in the prevention of diverse metabolic and cardiovascular diseases (**Angessa, 2006).** Cowpea has many beneficial uses as animal feed, human consumption, and income generation (**IITA, 2009).** In some cases, the pods are harvested when they are full-sized, just before they dry out, and then the grains are cooked and eaten as a vegetable. Cowpeas are important also as they improve the fertility of the soil in terms of nitrogen and phosphate (**Badoand and al, 2006).** Cowpea is the economic interest due to marketing of its products and by-production. In addition, the varieties early capable of producing in 55 days after sowing, often provide the first source of food the companion before any other harvest (**Timko and Singh, 2008**) and it doesn't require large inputs of fertilizer (**Quin, 1997).**

Cowpea (*Vigna uniguiculata)* would be an alternative culture for food security in Algeria, there for investigation of both agriculture profile and tolerance to abiotic stresses is important to maximize production and ensure food security in Algeria.

# *Stresses in plants*

## II. Stresses in plants

### 1. Abiotic stress:

In nature, plants are exposed to an ever-changing environment with increasing frequencies of multiple abiotic stresses (figure05). These abiotic stresses act either in combination or sequentially (**Anwar and al, 2021).** It constitutes a major factor, which affect the growth and yield formation of crop plants (**Canter, 2018; Zorb and al, 2019).** These abiotic stress including low or high temperature, deficient or excessive water, high salinity, heavy metals and ultraviolet radiation (UV) among other these stresses are posing a serve threat to agriculture and the ecosystem yield loss (**Wang and al, 2003; Wania and al, 2016).** About 90% of arable lands are prone to one or more of the above stresses (**Dos Reis and al, 2012)** which cause up to 70% loss in major food crops (**Mantri and al, 2012).**


**Figure 5 :** Abiotic stress sources affecting root and shoot growth of plants **(Raziye Kul and al, 2020).**

### 1.1. Definition of abiotic stress:

The abiotic stress is defined as the negative impact of non-living factors on living organism in a specific environment (**Najam W and al, 2014).** The abiotic stress include drought, salinity, low or high temperatures, cold, oxidative stress and heavy metal toxicity, where salinity and drought are the primary causes of crop loss worldwide (**Giora and al, 2012).**

## 1.2. Various types of abiotic stress:

### 1.2.1. Water stress (Drought):

The water stress caused by a water deficit constituting a permanent threat to survival plants, however, many of them could be drought tolerant by morphological, physiological and genetic adaptation to these unfavorable conditions that allow them to survive in regions with low rainfall and low soil water content (**Hopkins, 2003).**

### 1.2.2. Salt stress:

Salt stress is the exposure of plants to salinity, NaCl constituted the major component. Salt stress can appear in two forms, the first is gradual exposure to increasing level of NaCl while the second is exposing the plant to low level of salinity or it may be a combination of both forms. Salt shock is an extreme form of salt stress where plants are exposed suddenly to high level of salinity (**Yuri. S, 2013).**

## 2. Salinity:

### 2.1. Definition of salinity:

Salinization is a process of enriching a soil with salts soluble, which results in saline soil formation. Salinization can also be defined as a process accumulation of soluble salts (**Bouchoukh, 2010).** According to **Mermoud (2001),** soil salinization is the process of salt accumulation on the soil surface and in the root zone, which causes harmful effects on plants and soil. Salinity causes different types of stress including osmotic stress, ionic stress, oxidative stress and nutritional stress**. (Rasool and al, 2013)**.

### 2.2. Origin of soil salinity:

According to **Cherbuy (1991),** the Stalinization of an environment implies the presence of salts sources, which can be natural, called primary, and a salinization anthropogenic, generally linked to irrigation, which will be called secondary.

### 2.2.1. The natural source of soil salinity (primary salinity):

The salinity is said to be natural or primary, when the mineral salts which are the origin of this salinity comes from the saline groundwater or the alteration of the saline bedrock, and this

alteration is favoured by physico-chemical factors (wind, frost, thaw and often acid rain, loaded with $H_2CO_3$ (**Duchaufour and al, 1979).**

**Figure 6 :** Lake Wyara, Western Queensland, a naturally occurring saline lake **(Queen Sland, 2017).**

## 2.2.2. Anthropogenic source of salts (secondly salinity):

Salinization of secondary origin is induced by human activity, linked frequently to inappropriate farming practices. The main causes of this secondary soil salinization are:

- Use of poor-quality irrigation water and insufficient natural leaching.
- Rise of the underground water table close to the surface and transport of salts by capillary rise (**Marc, 2001).**


**Figure 7 :** Salinity cause by poor quality irrigation water (**Wang and al, 2018).**

## 3. The difference between salinization and sodization of soils:

The main differences between the sodic soils and saline soil are in the nature of anions and the pH of the soils. Studies demonstrate that carbonate or bicarbonate ions constituted the sodic soils with pH above 8,5, whereas chloride or sulphate ions dominates the saline soils with pH between 8,5; some plants grow well in salt affected coastal areas, shores of back waters lakes and marshy lands, the plants that cannot withstand even 10% of seawater are called glycophytes or non-halophytes (**Gorthan, 1995; Cherian and al, 1999; Parida and das 2005 ; Yavad and al, 2011, Mane and al, 2011).**

## 4. Distribution of saline soil in Algeria:

According to **Aubert (1975)** and generally "the sodic soils in North Africa come mainly from an action of the sea or from the presence of salty and gypsum lagoon deposits distributed in the stratigraphic scale from the Triassic the Quaternary.

In North Africa, salinization mainly affects irrigated areas and lower parts subject to high evaporation rates. In these regions, there is a significant risk of groundwater salinization (**Conacher and al, 1998).**

Sebekhas and chotts covered several thousand hectares in Algeria, where less than 100 years ago Mt. Atlas mastic tree were still cultivated (**Université de nice, 1992).**

Soils are widespread in Algeria, mainly in arid and semi-arid zones (**Durand, 1958),** works carried out by various authors show that salts **(Halitim and al, 1985)** affect the majority of agricultural soils in Algeria**.**

## 5. The plant and stress:

## 5.1. Perception of stress:

Plants present mechanisms for perceiving variations, it is this perception that triggers immune reactions in plants and sets in motion a cascade reaction that influence primary and secondary metabolisms as well as the expression of defence genes (**Bouchoukh, 2010).** The role of the perception of internal or external signals is to maintain a state balanced plant life and to protect against damage that can be generated or to facilitate the establishment of beneficial interactions for plants. (**Elmsehli, 2009).**

The stress would be perceived by membrane receptors at the level of the roots, which initiate a signal transmitted through a second messenger such as calcium, active oxygen species or ABA. These messengers act at target tissue level by activating phosphorylation cascades giving rise to the activation of genes involved in the stress response these genes allow the establishment of mechanisms for the survival of the plant: restoration of the osmotic balance, protection of membranes and proteins and detoxification by the elimination of active oxygen species **(Navarro, 2009; kroniewics, 2011).**

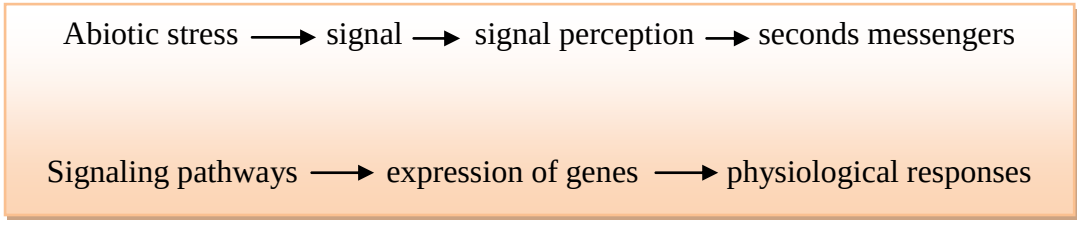



**Figure 8 :** Signalling in response to abiotic stress.

### 5.1.1. Role of calcium:

In plants, an ion seems to have a central role in the transduction of environmental signals: calcium. Indeed, rapid and transient changes in the cytosolic concentration of free calcium occur after the application of various stimuli in plants (**Knight, 1991 in Tafforeau, 2002**).

Calcium is a modulator of metabolism and development and serves as a second messenger in the translation of environmental stimuli in many organisms, especially plants. The walls of plant cells and the vacuole are storage sites calcium and plasma membranes and tonoplast have calcium channels (**Alvarez, 2004)**, Calcium is a major secondary messenger of stress signalling hydric (**Knight, 2000 in Lefebre, 2005).**

### 5.1.2. SOS pathway:

Different processes guarantee homeostasis; in the event of saline stress, a whole series of genes are particularly activated, encoding proteins responsible for the maintenance of Homeostasis: these are the SOS genes (Salt Overly sensitive).

Analysis of SOS mutants in A. thaliana allowed the identification of 3 proteins (SOS1, SOS2, SOS3), involved in the salt stress response. The process involving SOS begins after calcium binding to the $SOS_3$ protein that contains calcium and an N-myristilysis site (**Ishitani and al, 2000).** Modifications conformational caused by the binding of $Ca^{2+}$to $SOS_3$ will allow its

attachment to $SOS_2$. This serine / threonine kinase has an N-terminal catalytic kinase site and a very long C-terminal regulatory part (**Liu and al, 2000).**

Under normal conditions, the regulatory and catalytic sites interact with each other, preventing phosphorylation of a substrate because access to the catalytic site is blocked. The attachment of SOS3 to the regulatory part makes it possible to release the catalytic part (**Halfter and al, 2000).** The first target identified is SOS1, a Na + / H + antiport located in the plasma membrane, which would be activated following phosphorylation catalysed by the kinase of the SOS2-SOS3 complex (**Quintero et al, 2002) (**figure 9). The functional reconstitution of this pathway in *Saccharomyces cerevisiae* has verified that the calcium influx following stress saline triggered the SOS pathway (**Quintero et al, 2002).**

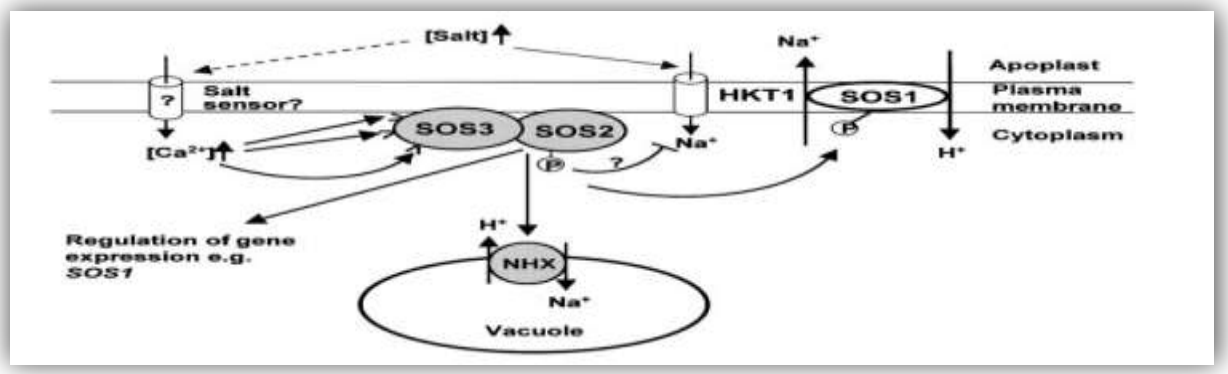



**Figure 9 :** Regulation of ionic homeostasis following salt stress according to **Chinnusamy and al, (2004).** Arrows indicate up regulation, lines down regulation.

## 5.2. The transcription of genes:

In addition to activating metabolic pathways such as SOS, the signals transmitted in the cell following stress will activate the transcription of genes allowing the cell to survive in hostile conditions. The intermediary of transcription factors does the activation of the transcription of these genes. These proteins attach to DNA at specific patterns and induce transcription of the gene downstream of this pattern (**Vincent, 2006).**

## 5.3. Expression of specific proteins:

At the end of the signalling cascade and following the activation of transcription, certain genes will be regulated in order to restore cell homeostasis and thus contribute to the resistance or adaptation of the cell to a given stress. These genes typically encode enzymes involved in the

production of molecules osmoregulators, transporters, detoxifying enzymes, chaperones (**Vincent, 2006).**

## 5.4. Classification of plants according to their tolerance to salinity:

### 5.4.1. Halophytic plants

According to **Tsope (1939**) in **Oudina (2014),** halophytic plants are divided into four classes (Figure 06):

**a) Obligatory halophytes**: These plants require salts throughout their life cycle.

**b) Preferred halophytes**: These are plants that require salts for their optimal growth, but they also exist in non-saline environment.

**c) Accidental halophytes**: These plants are found in saline environments by accident.

**d) Resistant halophytes:** These plants can develop in saline environments.

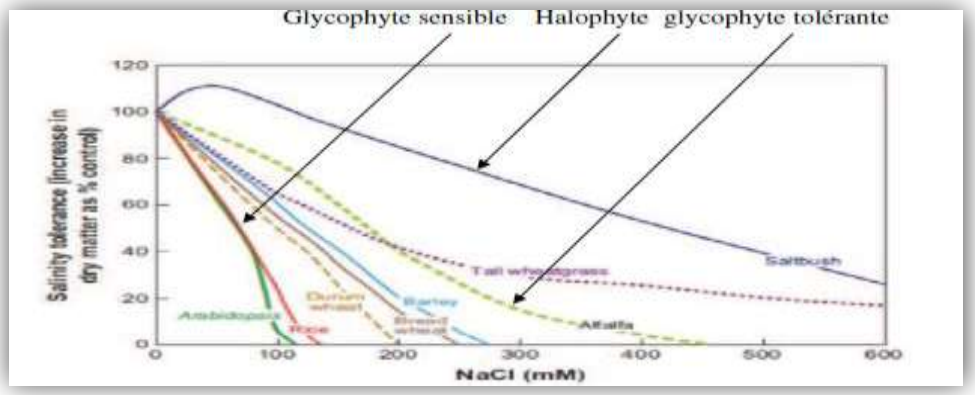



**Figure 10 :** Diversity of salt tolerance of various species (**Munns and Tester, 2008**)

### 5.4.2. Glycophytes plants:

From the Greek = gentle oppose halophytes. Cannot tolerate stress saline, and are severely disturbed or even killed by 100 to 200 mmol/l of NaCl (**Belkhodja, 2006 In Gaid, 2015).**

## 5.5. Physiological constraints imposed by salinity:

### 5.5.1. Ionic stress:

According to **Chinnusamy and al, (2004**) the accumulation of the toxic ions $Na^+$ and $Cl^-$ at the level of the mesophyll of the leaves, affects the growth and the metabolism of the plant where the salt damages the lipid and protein structures of the plasma membranes.

Presence of these ions disrupts cellular enzymatic activity mainly in photosynthetic tissues (**Hasegawa and al, 2000). Chinnusamy and al (2004)** see that ionic toxicities can be the result of the replacement of $K^+$ by $Na^+$ at the level of the active sites of proteins, also inducing a change in protein and enzymatic structures.

### 5.5.2. Nutritional stress:

Salinity is not a simple matter of high concentrations of Na + and Cl. Calcium, sulphate, carbonates may be present, with boron or selenium at excessive concentrations. At the same time, other nutrients, particularly phosphorus and nitrogen, may not be present or available in sufficient quantities to allow high growth rates (**Gorham, 1990).**

The increase in the concentration of $Na^+$ is accompanied by a reduction in the concentration of Mg, K, N, P and Ca in the plant. This nutritional imbalance is a possible cause of growth reductions in the presence of salts when essential ions such as $K^+$, $Ca^{2+}$ or $NO^{3-}$ become limiting (**Haouala and al, 2007).**

### 5.5.3. Osmotic stress (water stress):

The first consequence of salinization is the modification of the osmotic potential of the soil solution, when the salt content increases. According to **Song and al (2005),** the saltier the soil solution, the higher the osmotic pressure and the more it is difficult for the roots to extract water from the soil reserve. These results in a slowing of their growth. According to **Chinnusamy and al (2004),** the salt concentration depends on the water content of the soil and increases with high temperature; this is why the excess salts that affect plants are reached much faster in sandy soil than in clay soil which traps Na + ions via the negative charges of the clay.

## 5.5.4. Oxidative stress:

According to **Parent and al, (2008)** a consequence of environmental stresses, including salt stress, is the appearance of oxidative stress.

### 5.5.4.1. Definition:

ROS are present in the cell at reasonable doses; the balance between their rate of production and their rate of elimination regulates their concentration by antioxidant systems. Thus, in the quiescent state, the antioxidant / pro-oxidant balance (redox balance) is said to be in equilibrium. However, this redox homeostasis can be broken either by an excessive production of ROS or by a decrease in antioxidant capacities. We then speak of oxidative stress (**Camille and Mireille, 2011).**

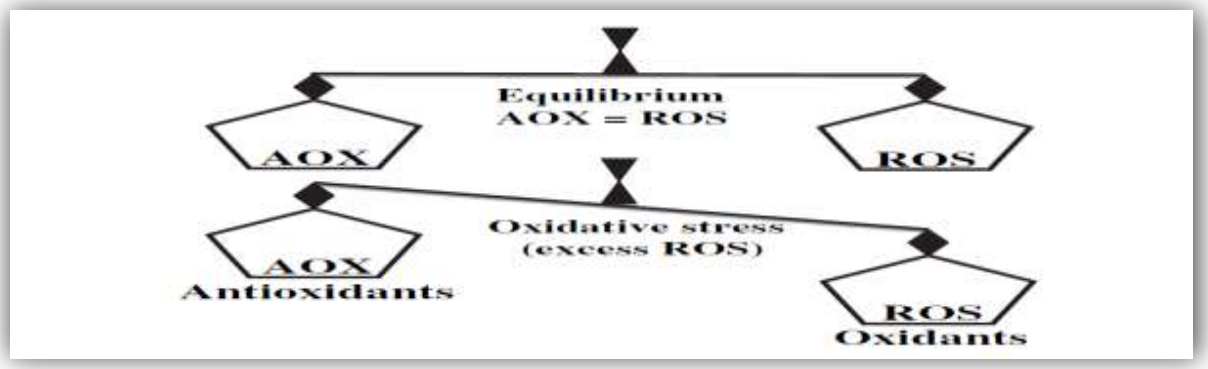



**Figure 11 :** Balance between ROS (Oxidants) and the antioxidant system
**(Gill and Tuteja, 2010)**

### 5.5.4.2. Oxidative stress markers:

### 5.5.4.2.1. Lipid peroxidation (Peroxidation of polyunsaturated fatty acids):

Membrane lipids are polyunsaturated fatty acids. These lipids are very sensitive because of their double bond, which can be easily attacked by ROS.

ROS cause oxidation of these polyunsaturated fatty acids, which results in the formation a large number of primary (Hydro Peroxides), and secondary (Aldehydes) products.

This lipid peroxidation phenomenon tends to make fatty acids more hydrophilic which will alter the structure and function of cell membranes, in particular by increasing their

permeability to substances that are not normally able to cross them (**Moller and al, 2007; Gill and Tuteja, 2010).**

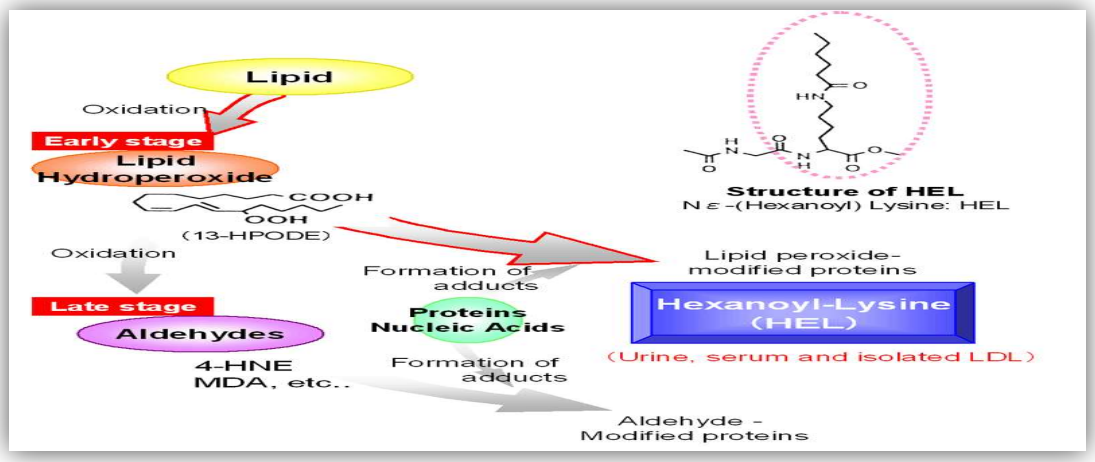



**Figure 12 :** Different steps of lipid peroxidation (**Hanaa and al, 2012).**

## 5.5.4.2.2. System antioxidants:

System antioxidants are redoxes agents, which react with oxidants and either stop, or slow down oxidation processes (**Leopoldinin and al, 2011).**

Reduced oxygen compounds have a very extensive chemistry. They are the source of mutagenic effects and cause alterations in proteins and lipids.

To cope with these drawbacks. Living cells are equipped with several effective weapons to eliminate these ROS. These mechanisms can be divided into two categories depending on whether they involve enzymes in a direct or indirect way**. (Sofo and al, 2004).**

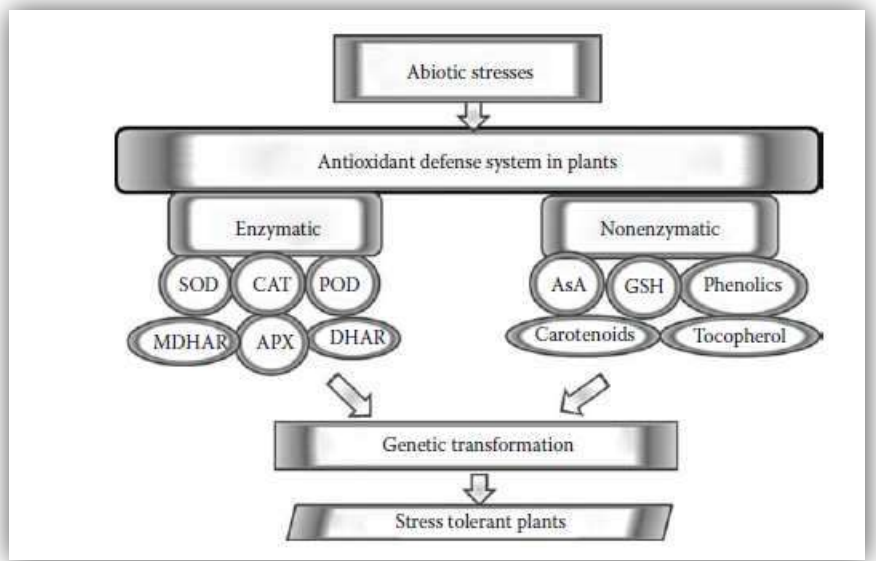



**Figure 13 :** Antioxidant defence system: enzymatic components and not enzymatic **(Joseph and Jini, 2011)**

## 6. Mechanisms of plant tolerance or adaptation to salt stress:

**Shannon and Griene (1999)** as the inherent ability of plants to resist the effects of high salt concentrations in the root profile on leaves without experiencing significant adverse effects define plant resistance to salinity. Several mechanisms contribute in the tolerance to salinity (**Chen and al, 2008). Leuitt (1980)** distinguished between avoidance and tolerance mechanisms, and used the term salinity resistance to refer to a combination of tolerance and avoidance strategies.

Avoidance mechanisms include delayed germination or maturity until the initiation of preferential root growth in non-saline soils (**Schulze and al, 2005),** compartmentalization and secretion of salts by specialized organs such as salt glands and salt hairs or the storage of salts in old leaves (**Klein and al, 2008).**

The salt response of plant species depends on the species itself, its variety, salt concentration, growing conditions and stage of plant development (**Poljakof Mayber, 1975).** In plants sensitive to NaCl, Na accumulates in the roots but is excluded from the leaves. These plants are said to "exclude" with respect to Na. Conversely, NaCl tolerant plants are said to be "includer" because they generally have more Na loaded leaves than roots when grown in the presence of salt (**Faouzi and al, 2007).**

To limit salt stress, plants trigger tolerance mechanisms that contribute to adaptation to stress and which have in order: Physiological, morphological and biochemical adaptation.

## 6.1. Physiological adaptation:

### 6.1.1. Exclusion of ions:

In plants sensitive to NaCl, Na + accumulates in the roots and then excluded from leaves, these plants are called "excluders" **(El Madidi and al, 2003).** The plant prevents salt from reaching the leaves. A first barrier exists at the level of the endoderm the inner layer of the cells of the root, as well as the selective transport makes it possible to adsorb the useful nutrient ions and to re-excrete the Na + ions (**Genoux and al, 1991).**

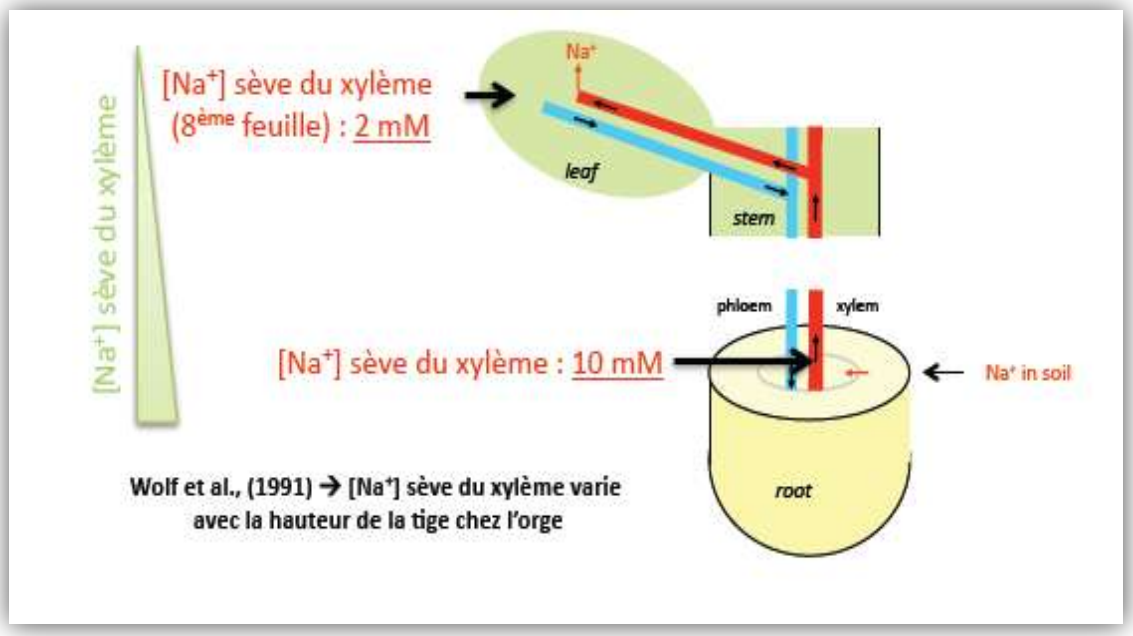



**Figure 14 :** $Na^{2+}$concentration varies depending on the length of the stem of plan**t (Wolf and al, 1991).**

### 6.1.2. Inclusion:

The plants "includer" resistant to NaCl, accumulated $Na^+$ in the leaves where it is sequestered either in the vacuole, the leaf epidermis, the aged limbo, vacuoles are closed compartments within the cell, the salt is thus isolated from the cellular constituents vital, or excreted by glands to the outside. (**Berthomieu and al, 2003).**

Excretion in the salt glands is very specific; first $Na^+$, $Cl^-$ and $HCO^-_3$ are excreted against the concentration gradient, while ions like $Ca^{2+}$, $No_3^-$, $SO_4^-$ and $H_2PO_4^-$ are held against their gradient (**Hopkins, 2003).**

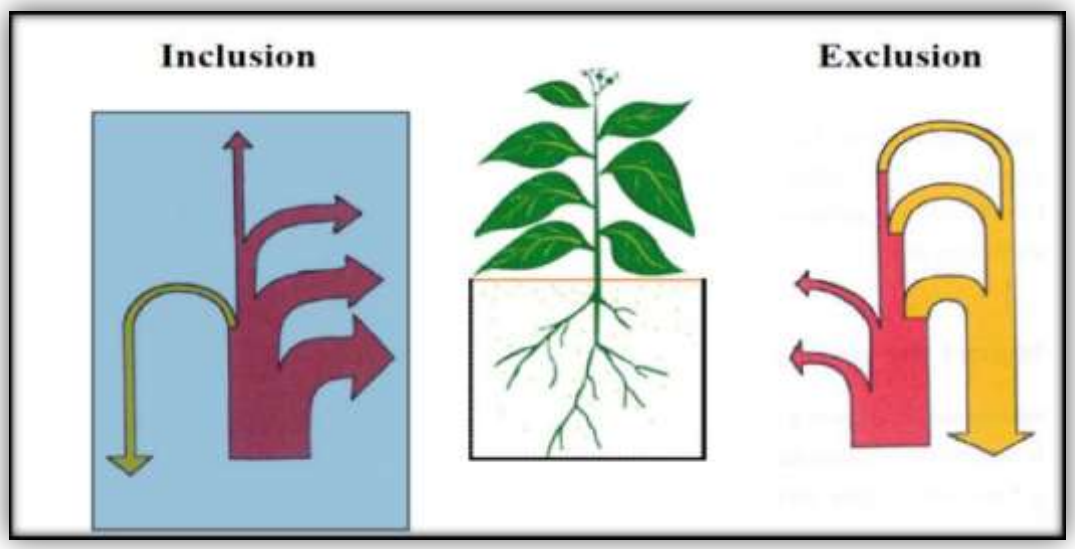



**Figure 15 :** Illustration of "inclusion" and "Exclusion" strategies

**(Aurélie Levignon and al, 1995).**

The "inclusion" strategy characterizes the fact of promoting sodium storage in the leaves while preserving the apical meristem while the strategy "Exclusion" characterizes the fact of promoting the recirculation of Na to the roots.

## 6.1.3. Vacuolar compartmentalization:

Compartmentalization of ions between organs (roots / aerial parts), tissues (epidermis / mesophyll), or between the cell compartments (vacuole / cytoplasm) is one of the adaptation mechanisms to salt stress (**Ouerghi and al, 1998)**. The plant in fact uses salt to adjust the osmotic pressure of its cells. It captures the salt that reaches the leaves, just like water, by the upward movement of the sap in the vessels. Inside the cells, the salt is then stored in the vacuoles using molecular "pump" systems. Vacuoles being closed compartments within the cell; the salt is thus isolated from the constituent's vital cells (**Sentenac and Berthomieu, 2003**).

### 6.1.4. Induction of plant hormones:

Physiological responses to various stresses such as drought or excess water, salinity, soil compaction and root hypoxia have been shown to have similar characteristics (**Schulze and al, 2005).** They all cause an increase in abscisic acid (ABA) in the aerial part or reduction in cytokinin concentrations (**Gregory, 2005; GUO and Dauidho, 2008).**

The high concentration of salt triggers an increase in the levels of plant hormones (**Parida and Das, 2005)**. ABA is responsible for the alteration of genes induced by salt stress. During salt stress, there is an increase in the production of ABA at ethylene (**Gomez and al, 2002)**. It turned out that ABA alleviates the inhibitory effect of Na Cl on photosynthesis, growth and tronslocation of assimilates (**Popova and al, 1995**). ABA promotes stomata closure by changing the flow of ions in guard cells under saline stress conditions.

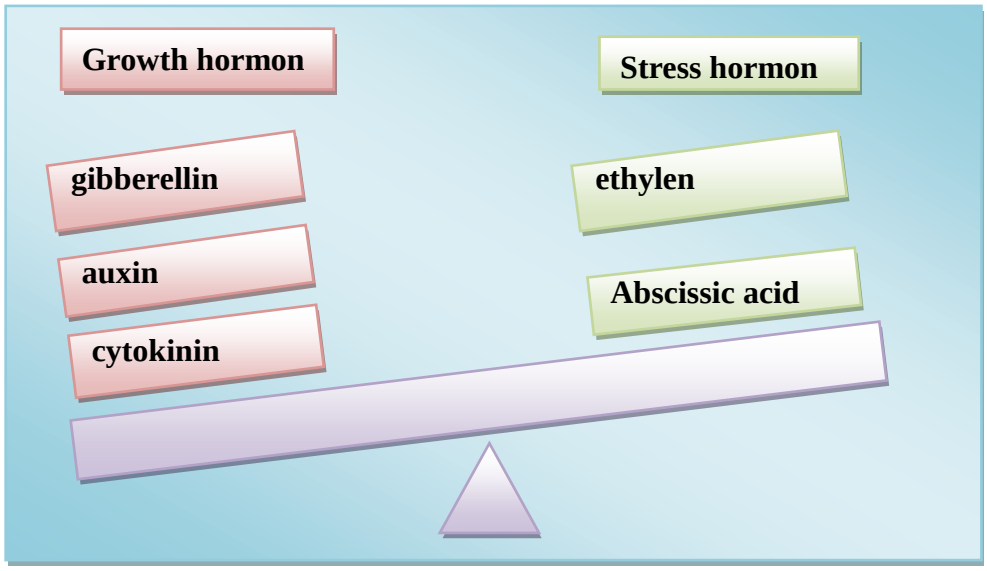



**Figure 16 :** Hormones produced by the plant during stress

**(Stoller europe, 2019).**

## 6.2. Biochemical adaptation:

Faced with salt increase in the soil, an osmotic adjustment may occur, but to varying degrees in most plants. One of the main characters physiological tolerance to environmental constraints is the osmotic adjustment (**Doumi, 2015).**

Osmotic adjustment or osmoregulation is a major adaptation mechanism for resistance to osmotic stress, which is expressed by the ability of a plant to active accumulation; it is generally considered an important element in the tolerance of plants to salt stress. This adjustment involves the accumulation, at the level cellular, organic solutes such as soluble sugars (fructose, glucose, raffinose, etc.) and certain amino acids for example: proline (**Rabiaa, 2019).**

## 6.2.1. Accumulation of proline:

Proline ($C_5H_9NO_3$) accumulates greatly in plants exposed to salt stress (**Weigel and al, 1990).** The accumulation of proline in various stressed plant species has been correlated with their ability to tolerate, and its concentration is generally higher in tolerant plants than sensitive plants, for which the accumulation of proline seems rather to be a simple reaction of the plant than a behaviour of adaptation and stress tolerance (**Hanana and al, 2011**).

Proline can act as an osmolyte, ROS scavenger and molecular chaperone, prostabilizing protein structure, thus protecting cells from damage caused by stress**. (Nahila Afaf, 2016).**

### 6.2.1.1. Biosynthetic proline:

Glutamic acid is easily converted into proline. First, the γ carboxyl group is reduced to the aldehyde, yielding glutamate semialdehyde. The aldehyde then reacts with the α–amino group, eliminating water as it forms the Schiff base. In second reduction step, the Schiff base is reduced, yielding proline.

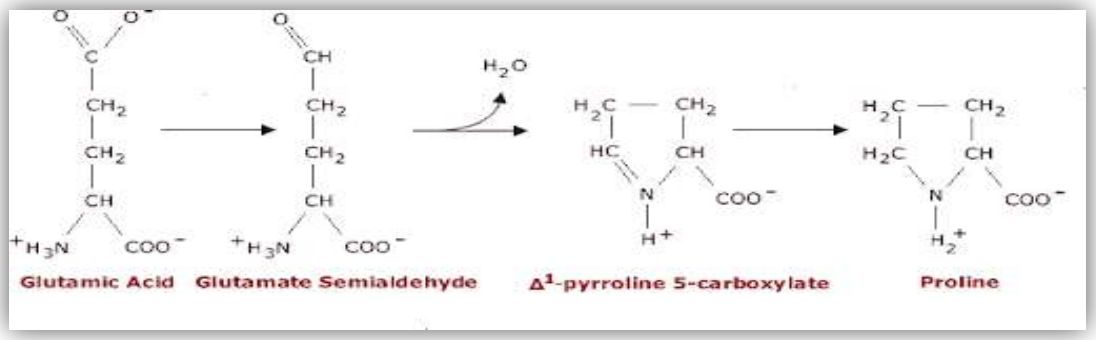



**Figure 17 :** Biosynthetic of proline**. (The Biology Project, 2003).**

### 6.2.1.2. Role of proline:

According to **Szabados and Savouré (2010),** Proline has many functions in plants:

- Protein synthesis.
- Osmolyte.
- Maintenance of redox power.
- Development regulation.
- Actor in the metabolic signalling network controlling mitochondrial functioning.

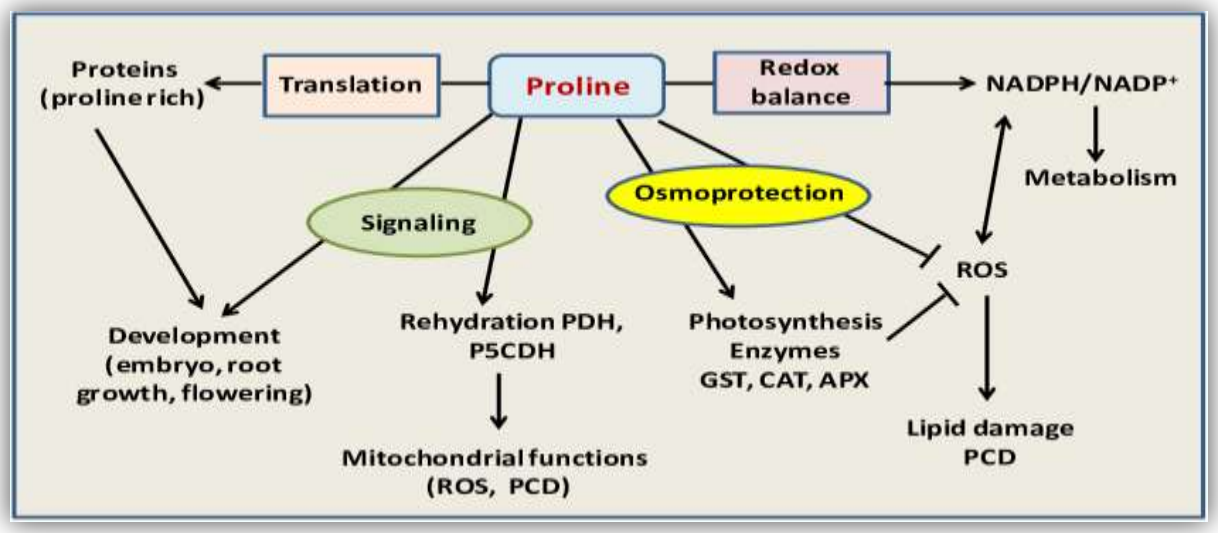



**Figure 18 :** Multifunctional roles of proline in plants to mitigate deleterious effects of salinity stress as well as in protein synthesis and plant development. (**Faiçal Brini, 2020).**

## 6.3. Morphological adaptation:

Salinity is known to induce many changes in morphology and physiology of plants. The morphology and structure of the latter are adapted in the sense of water saving (**Asloum, 1990; Heller and al, 1998**). The characters associated with this adaptation are:

- ✓ The presence of a thick cuticle.
- ✓ Rare stomata or closure of the stomata.
- ✓ Very developed roots.
- ✓ A decrease in leaf area.
- ✓ Weak branching, decrease in diameter length, dry weight of stems, roots.
- ✓ A shortening of the internodes and a reduction in the number of nodes.
- ✓ Loss of turgor of cells.

## 7. Effect of salinity on plants:

### 7.1. Effect on germination:

The presence of excess salt in the soil is one of the critical factors affecting unfavourably germination of the seed, preventing species from adapting to saline environments (**Sosa and al, 2005)**.

The germination of plants whether halophytes or glycophytes is affected by salinity. Depending on the species, the depressive effect can be osmotic or toxic in nature (**Ismail, 1990).**

### 7.1.1. Osmotic effect:

Salinity inhibits the absorption of water, the mobilization of reserves and their transport to the embryo. However, the embryo must reach a critical hydration threshold before start of germination processes.

### 7.1.2. Toxic effect:

The toxic effects are linked to a cellular accumulation of salts, which cause disturbances of the enzymes involved in the physiology of germinating seeds, prevent the breaking dormancy of embryos and lead to a decrease in the germination capacity. **Rejili and al, (2006**) report that good seed germination and emergence under stress saline is a valid criterion to ensure adequate establishment in soils affected by salt. However, **Ben Ahmed (1996)** reports that the correlation between tolerance at the stage of seed germination and plant tolerance during other growth periods is not mandatory.

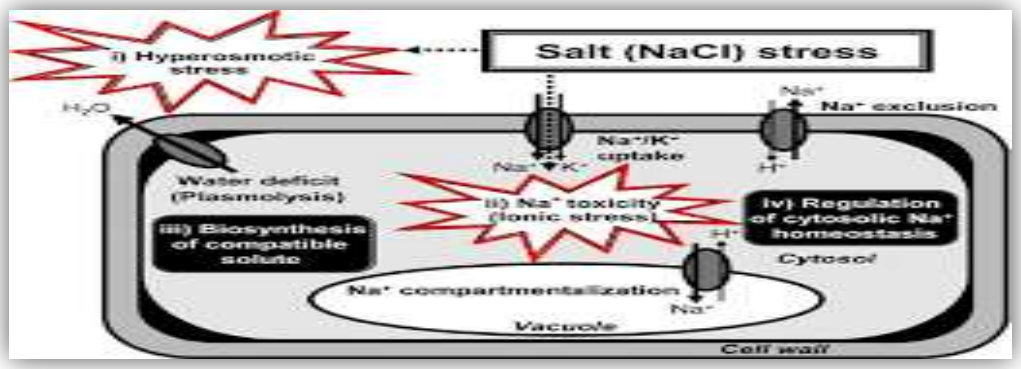



**Figure 19 :** Toxic effects of NaCl on the plant (**Jabnoune, 2008)**.

# *Chapter II:*

# *Materials and Methods*

## II. MATERIALS AND METHODS:

In this study, we conducted an experiment in the Laboratory of Natural Sciences and Materials of university of Mila, which concerns the effect of NaCl on the germination of 11 seeds genotypes of cowpea (*Vigna unguiculata subsp. unguiculata (L.) Walp).*7 genotypes from different regions of Algeria and 4 foreign genotypes, subjected to salt constraints (NaCl) during 5 days of stress.

### 1. Plant Material:

The plant material consists of 11 genotypes of *Vigna unguiculata (L.)* described in Table 3.

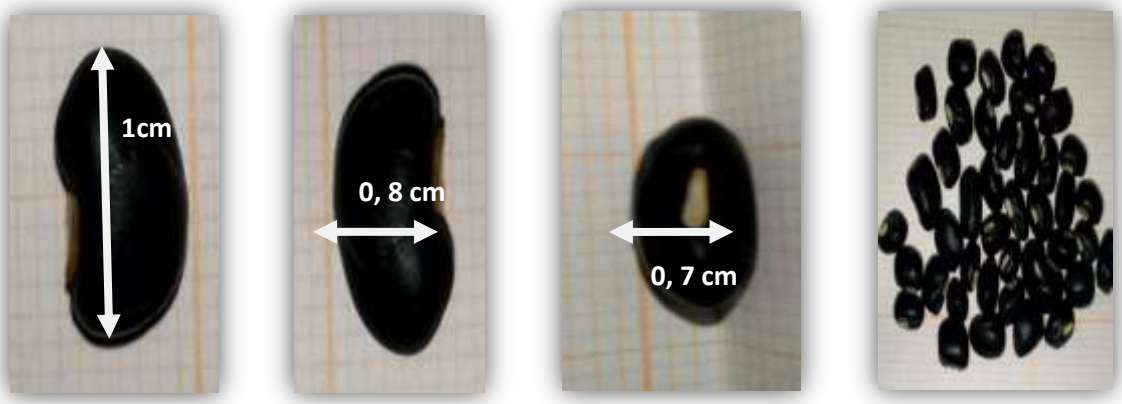
**Table 3 :** Agro-morphological characteristics of cowpea ecotypes studied.

| **Population / characteristics Agro morphological** | **Abbreviation** | **colour of seeds** | **weight of 100 seeds** | **origin** |
|---|---|---|---|---|
| Black Tadleghat | TT | black with white spot | 8. 87 | Timimoun |
| Vigna Arabia | E1 | Brown with black spots | 8. 69 | Taref |
| Ilizi | I1 | Beige with black spots | 22, 48 | Ilizi |
| Oued | O | Beige with black spot | 21, 53 | Oued |
| Tizi Ouzou | TZ | Beige with black spots | 31, 85 | Tizi ouzuo |
| Azazga | AZ | Beige with black spots | 23, 84 | Azazga |
| Rouge Aoulef | RA | Clear brown with beige spot | 12, 20 | Unknown |
| Néibé d'Asie | NA1 | Red with brown spot | 14, 43 | Asie |
| Néibé d' Australia | NA2 | Red with brown spot | 10, 72 | Australia |

| | | | | |
|---|---|---|---|---|
| Néibé Brasil | NB | Beige with black spots | 22, 72 | Brezil |
| Néibé Egypt | NE | Beige with black spots | 20, 29 | Egypt |

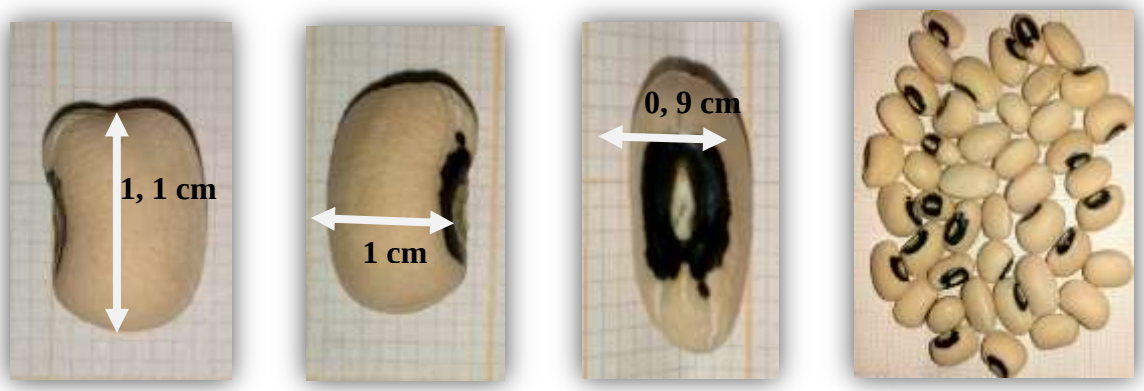
The seeds of nine cowpea genotypes (Azazga, Vigna Arabia, Rouge Oulef, Néibé d' Australia, Noir Tadleghat, Néibé Brazil, Néibé Egypt, Néibé d'Asie, Tizi Ouzou) which were used for this study, were provided by ITCMI (technical institute for market gardening and industrial crops) of STAWALI and the seeds of cowpea genotypes ( cowpea of Oued and Ilizi) were provided locally.

## 1.1. Seeds morphology:

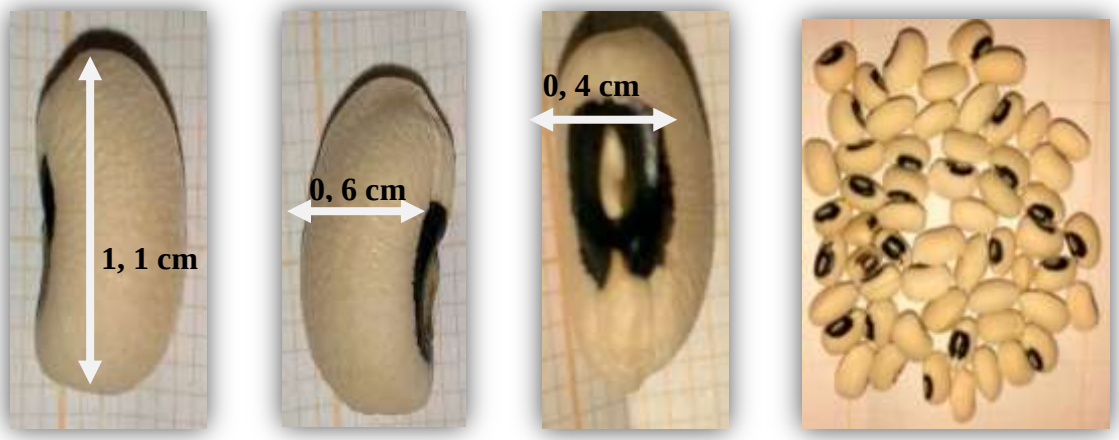



**Picture 1:** Seeds of cowpea varieties TT.

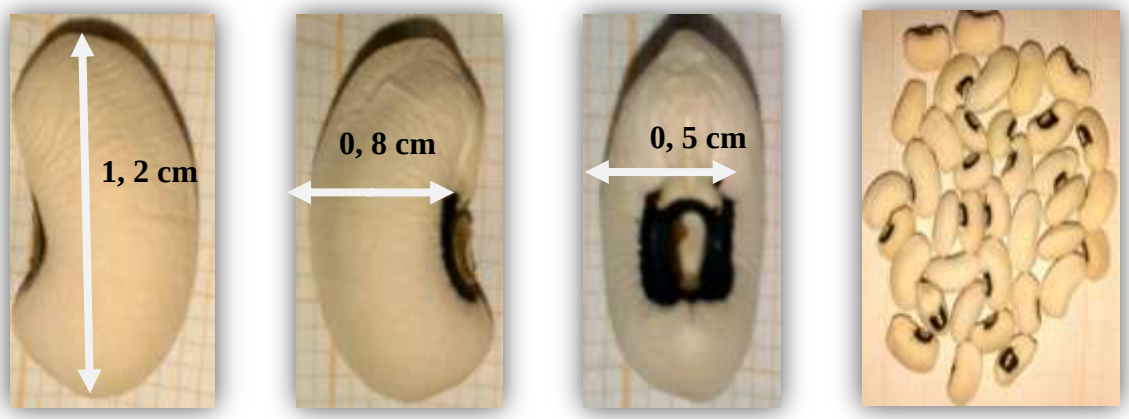
**Pictur 2:** Seeds of cowpea, varieties E.



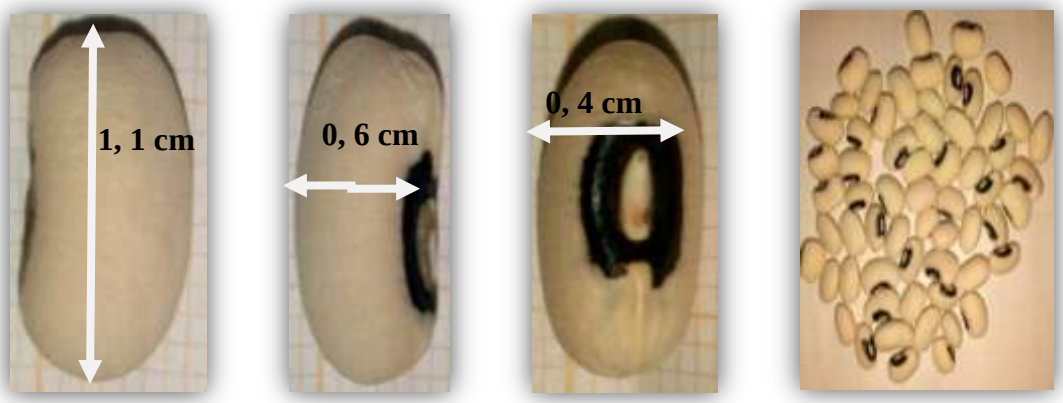

**Picture 3:** Seeds of cowpea varieties I.



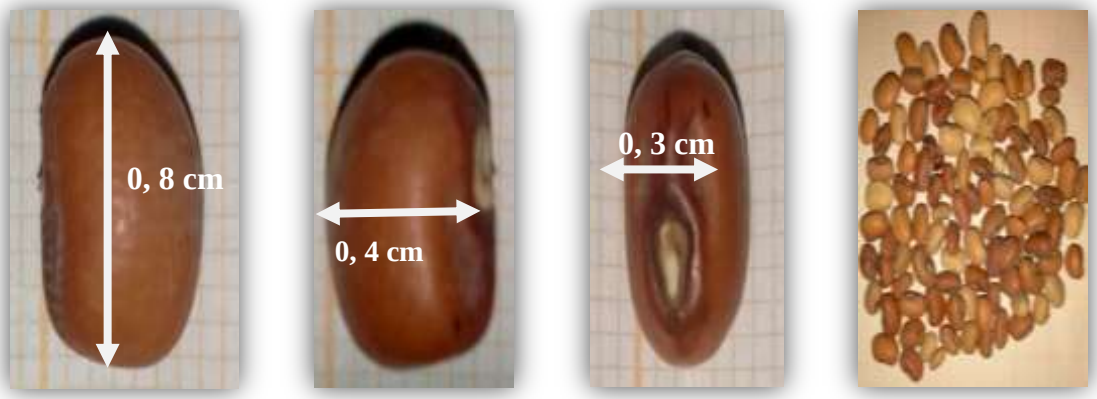

**Picture 4:** Seeds of cowpea, varieties O.



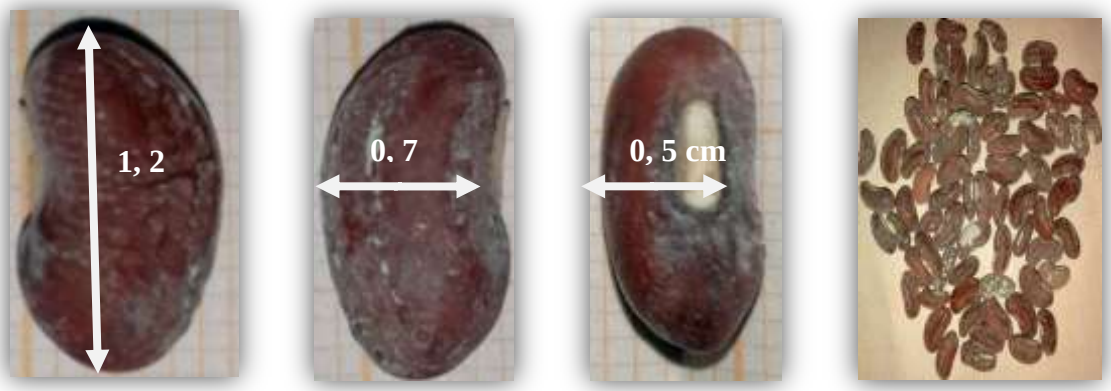

**Picture5:** Seed of cowpea, varieties TZ.



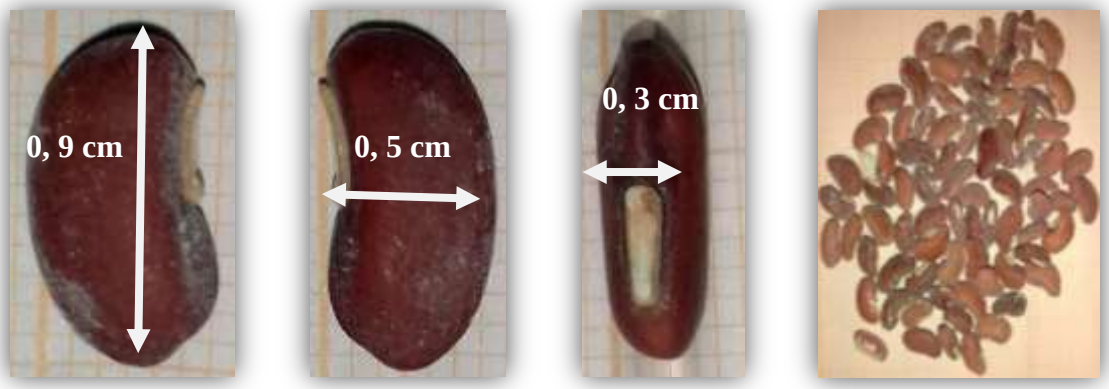

**Picture 6:** Seed of cowpea, varieties AZ.



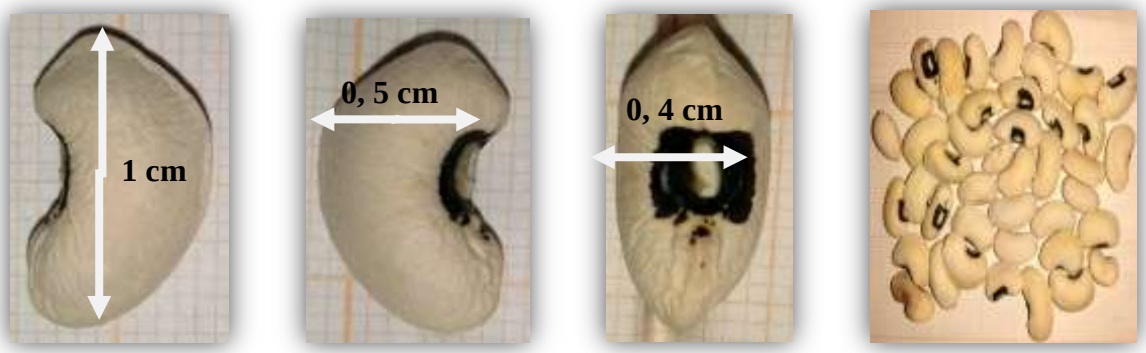

**Picture7:** Seed of cowpea, varieties RA.



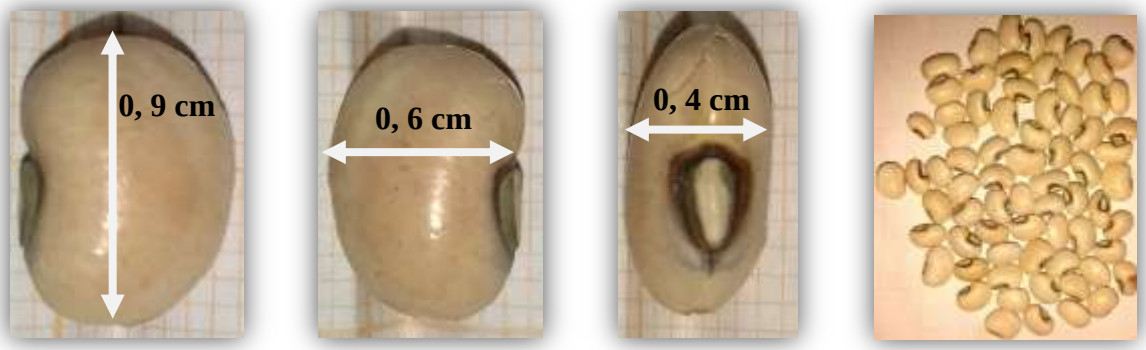

**Picture8:** Seed of cowpea, varieties NA1.




**Picture 9 :** Seed of cowpea, varieties of NA2.




**Picture 10:** Seeds of cowpea, varieties NB.




**Picture 11:** Seeds of cowpea varieties NE.

## 2. Experimental protocols:

### 2.1. Germination under salt stress

### 2.1.1. Seed preparation:

For each genotype, seeds numbering 90, i. e. 3 repetitions of 30 seeds per Petri dish are disinfected with bleach 5%, and then rinsed with distilled water several times. This experiment was conducted in laboratory at temperature 25 C°(natural sciences and materials laboratory of university of Mila).

**Figure 20 :** Germination experiment of the 11 cowpea genotypes studied.

### 2.1.2. Preparation of saline solution:

Four level of salinity solution of 0, 50, 100, 150 mmol/L of NaCl was used for creating salt stress.

The dissolved calculated amount of NaCl in tap water was used to make saline solution 50, 100, 150; the tap water was used as control (0mM)

### 2.1.3. Application of salt stress:

Salt stresses is applied to the seeds, it induced by different concentration of NaCl (0, 50, 100, and 150). The control seeds are watered by distilled water and 50, 100, 150 (mmol/L) solution for the stressed seeds. The required amount of salt solution was irrigation as per necessary.

## 2.1.4. Germination parameters:

The parameters studied during this work are:

### 2.1.4.1. Germination rate:

This is the daily percentage of maximum germination or daily germination rate obtained in conditions chosen by the experimenter; it depends on the conditions of germination and treatments previously undergone by the seeds (**Mazliak, 1982).**

Germination rate is calculated according to the following equation:

$$Percentage\ of\ germination = \frac{Number\ of\ seed\ germinated\ at\ final\ count}{Number\ of\ seed\ placed\ for\ germination} \times 100$$

### 2.1.4.2. The germination kinetics:

To better understand the meaning physiological germination behaviour of cowpea populations studied, the number of germinated seeds was counted daily up to the 5th day.

This kinetics is established from the cumulative rate of germinated seeds, i. e. the variation in germination rates as a function of time expressed in days.

### 2.1.4.3. Early germination:

In general, each species has an early germination specific to its nature. Because even placed in same experimental conditions, the beginning of the radicle to appear through Integuments will not occur at the same time in all seeds (**Come, 1975).** In this case, the earliness of germination is expressed by the rate of first germinated seeds corresponding to the time interval between sowing seeds and the first germinated seeds (**Belkhodja, 1996).**

## 2.1.5. Biometric parameters:

### 2.1.5.1. Shoot length:

Seedling from each Petri dish was collected as a sampling after placement for germination at 5 days. The shoot length (Cm) of individual seedling was recorded manually with scale.

### 2.1.5.2. Dry weight of grains:

An electric even was for drying the grains at 75c° for 72 hrs and weights were recorded with an electrical balance.

### 2.1.5.3. Vigour index:

Was calculates by using the formula of **Abdul Baki and Anderson (1973)**, as shown below:

Vigor index (VI) = germination (%) × ( mean shoot length + mean root length)

### 2.1.6. Statistical analyses:

The statistical significance of the results during this study was tested by two-way analysis of variance (ANOVA) of cultivar.

## 2.2. Seedling growth under salt stress:

### 2.2.1. Seedling preparation:

The seeds were washed with 5% of bleach for 5 min then rinsed with distilled water several times.

We Sow carefully in pots filled with industrial peat (black peat) watering with distilled water is carried out regularly.

The experiment was carried out in the natural sciences and materials laboratory of university of Mila.

### 2.2.2. Application of saline treatment:

When the first three leaves appear, we start the saline treatment, each group of different varieties of cowpea having undergone a specific stress based on NaCl solution (0, 50, 100, 150) for 15 days.

## 2.3. Proline Determination with colorimetric method:

To determine the level of proline in the plant, we followed the following steps:

1) We collected the samples, we measured their fresh weight, and we used 100 mg for reaction. Then we stored the samples at -50 C°.

2) We add 3 % of sulfosalicylic acid (5 µL/mg fresh weights), we grind the plant material. In addition, we keep the tubes on ice until finishing with all samples.

3) We centrifuge the samples for 5 min at centrifuge with maximum speed.

4) We prepare the reaction mixture in a separate tube: glacial acetic acid, 200 µL acidic ninhydrin.

5) We add 100 ul from the supernatant of the plant extract, we mix the tubes well, to avoid high pressure and accidental opening of the tubes in subsequent reaction, we puncture the lid of micro centrifuge tube with a needle.

6) We incubate the tubes at 96 C° for 60 min.

7) We put the tube on ice for terminate the reaction.

Finally, we extract the samples with toluene: we add 1 mL toluene to the reaction mixture, we vortex the samples for 20 second, we leave them on the bench for 5 min to allow the separation of the organic and water phases. (We must use the gloves and mask in this experience).

# *Chapter III:*

# ***Result and discussion***

# 1. Results:

## 1.1. Germination rate:

**Table 4 :** Means of germination rate of genotypes in First day.

| | S0 | S1 | S2 | S3 |
|---|---|---|---|---|
| **TT** | 4, 33 | 0, 00 | 16, 67 | 0, 00 |
| **E** | 0, 00 | 0, 00 | 3, 67 | 0, 00 |
| **I** | 1, 33 | 10, 00 | 15, 33 | 6, 00 |
| **O** | 12, 67 | 9, 00 | 29, 67 | 9, 33 |
| **TZ** | 2, 33 | 0, 00 | 29, 67 | 0, 00 |
| **AZ** | 7, 00 | 0, 00 | 29, 67 | 0, 00 |
| **RA** | 3, 67 | 0, 00 | 21, 33 | 0, 00 |
| **NA1** | 4, 67 | 0, 00 | 10, 33 | 0, 00 |
| **NA2** | 4, 67 | 0, 00 | 10, 33 | 0, 00 |
| **NB** | 8, 67 | 3, 67 | 7, 67 | 0, 00 |
| **NE** | 9, 00 | 0, 00 | 3, 00 | 0, 00 |

S0: Control, S1:50mmol/l, S2:150mmol/l, S3 150mmol/l.

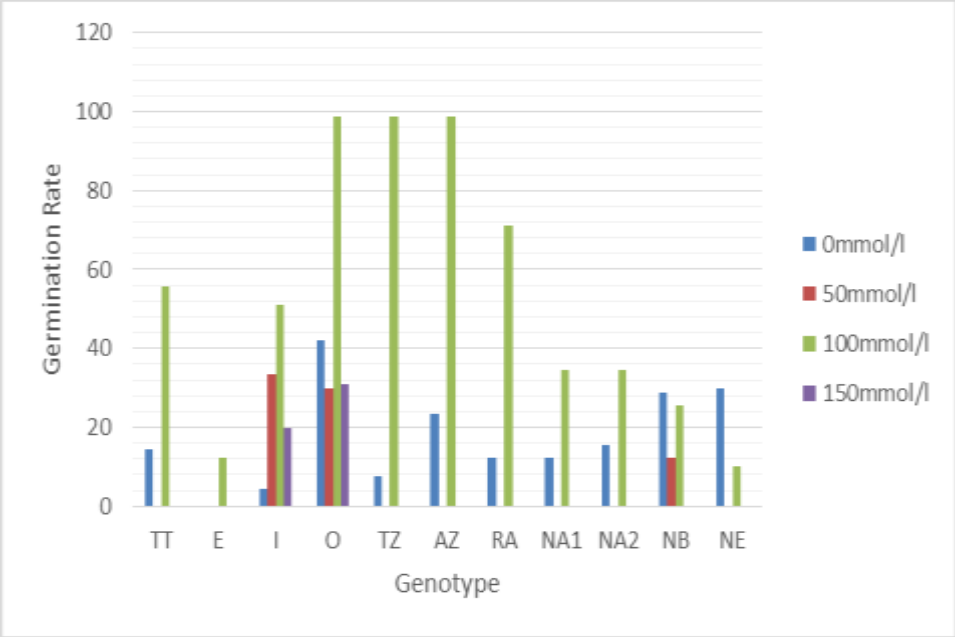



**Figure 21:** The germination rate of 11 genotype of cowpea in the first day.

For the germination rate, after one day we noted that the genotypes of (O), (AZ), (TZ), were 100 % germinated in 100mmol/l, while the varieties of (E) and (NE) have the less germination rate, which appreciate, by 10%. The varieties of (RA), (NA1), (NA2), (NB) and (TT) have average rate germination.

**Table 5 :** Means of germination rate of genotypes in third day.

| | S0 | S1 | S2 | S3 |
|---|---|---|---|---|
| **TT** | 15, 67 | 12, 00 | 0, 00 | 1, 33 |
| **E** | 9, 33 | 11, 00 | 0, 00 | 1, 00 |
| **I** | 30, 00 | 29, 67 | 5, 67 | 15, 33 |
| **O** | 30, 00 | 30, 00 | 10, 00 | 12, 33 |
| **TZ** | 30, 00 | 18, 00 | 13, 33 | 19, 33 |
| **AZ** | 30, 00 | 23, 67 | 5, 33 | 17, 33 |
| **RA** | 26, 00 | 16, 67 | 0, 00 | 1, 00 |
| **NA1** | 9, 67 | 10, 67 | 0, 00 | 1, 00 |
| **NA2** | 19, 33 | 15, 00 | 0, 00 | 0, 00 |
| **NB** | 14, 33 | 12, 33 | 0, 00 | 9, 67 |
| **NE** | 1, 33 | 1, 00 | 2, 33 | 2, 33 |

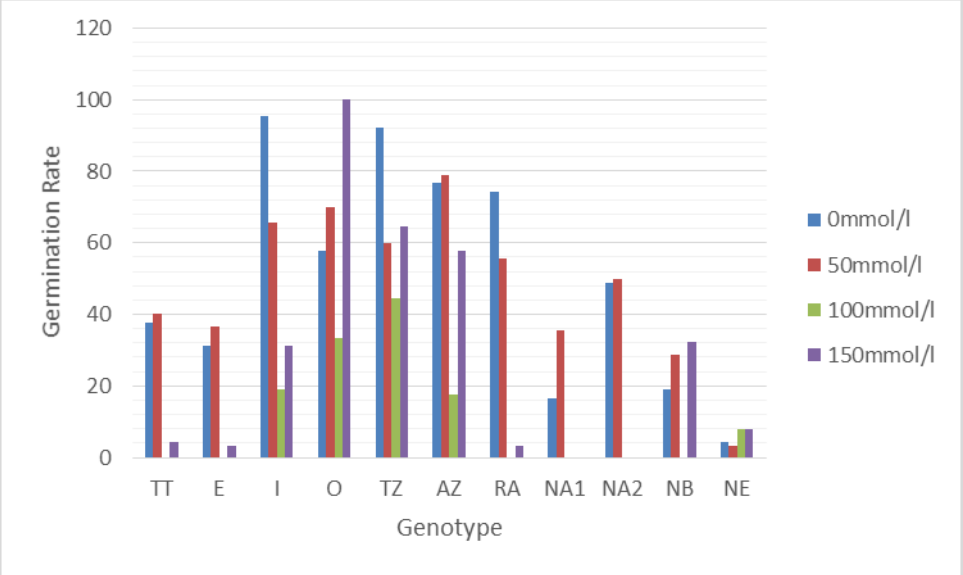



**Figure 22 :** The germination rate of 11 genotype of cowpea in third day.

In the third day, we observed that the varieties (O), (AZ) and (TZ) had the highest germination rate for the concentration 50 and 150 mmol / l, and a total absence of germination in varieties (NA1) and (NA2) in 150mmol/l, but in the concentration 150 mmol/l we observed that (RA), (TT), ( E) and (NE) were the weakest, and average germination rate for (RA) and (NA2) in the concentration 100 mmol /l.

(NE) is considered as the weakest genotype in terms of germination rate in different saline concentrations (0mmol/l, 50mmol/l, 100mmol/l, and 150mmol/l).

**Table 6 :** Means of germination rate of genotypes in fifth day.

| | S0 | S1 | S2 | S3 |
|---|---|---|---|---|
| **TT** | 21, 33 | 21, 33 | 21, 33 | 21, 33 |
| **E** | 10, 33 | 10, 33 | 10, 33 | 10, 33 |
| **I** | 30, 00 | 30, 00 | 30, 00 | 30, 00 |
| **O** | 30, 00 | 30, 00 | 30, 00 | 30, 00 |
| **TZ** | 30, 00 | 30, 00 | 30, 00 | 30, 00 |
| **AZ** | 30, 00 | 30, 00 | 30, 00 | 30, 00 |
| **RA** | 27, 00 | 27, 00 | 27, 00 | 27, 00 |
| **NA1** | 27, 33 | 27, 33 | 27, 33 | 27, 33 |
| **NA2** | 15, 67 | 15, 67 | 15, 67 | 15, 67 |
| **NB** | 18, 33 | 18, 33 | 18, 33 | 18, 33 |
| **NE** | 1, 67 | 1, 67 | 1, 67 | 1, 67 |

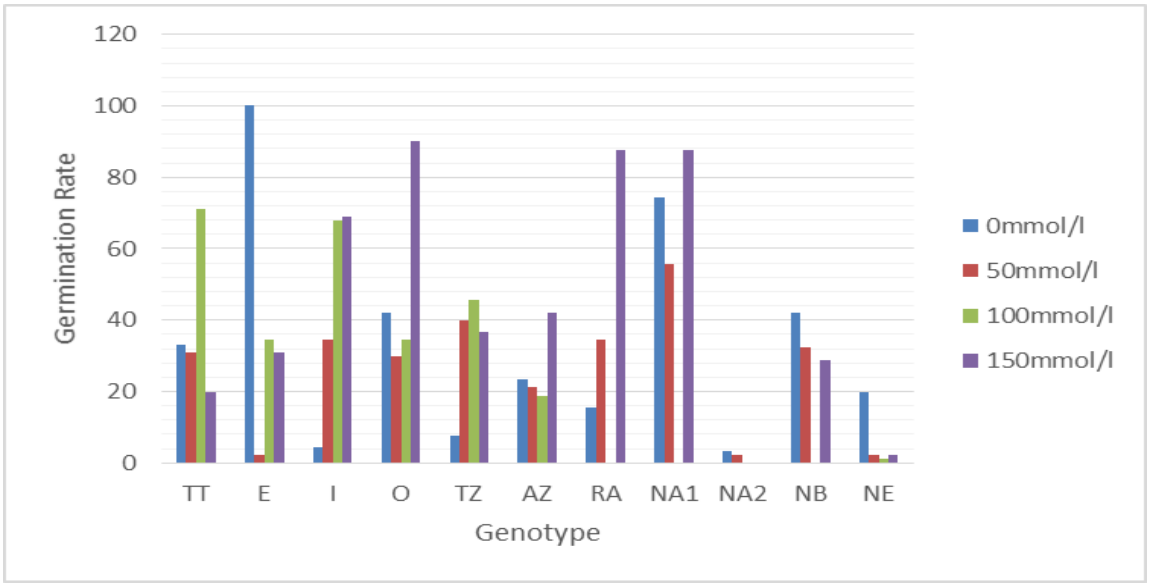



**Figure 23 :** Germination rate of 11 genotypes of cowpea in fifth day

There is an increase in the germination rate for the varieties of (O), (I), (RA), (NA1) in the concentration 150 mmol / l around 90 %, and a remarkable increase of germination rate for (TT), (I) in the concentration 100 mmol/l around 71, 7 %. In other hand, the genotypes (TZ), (AZ) in all concentrations were average, but (NA2) and (NE) were almost none.

## 1.2. Early germination:

**Table 7 :** Means of early germination of genotypes of cowpea.

| genotype | DAY1 | DAY3 | DAY5 |
|---|---|---|---|
| **TT** | 4, 33 | 15, 67 | 21, 33 |
| **E** | 0 | 9, 33 | 10, 33 |
| **I** | 1, 33 | 30 | 30 |
| **O** | 12, 67 | 30 | 30 |
| **TZ** | 2, 33 | 30 | 30 |
| **AZ** | 7 | 30 | 30 |
| **RA** | 3, 67 | 26 | 27 |
| **NA1** | 4, 67 | 9, 67 | 27, 33 |
| **NA2** | 4, 67 | 19, 33 | 15, 67 |
| **NB** | 8, 67 | 14, 33 | 18, 33 |
| **NE** | 9 | 1, 33 | 1, 67 |

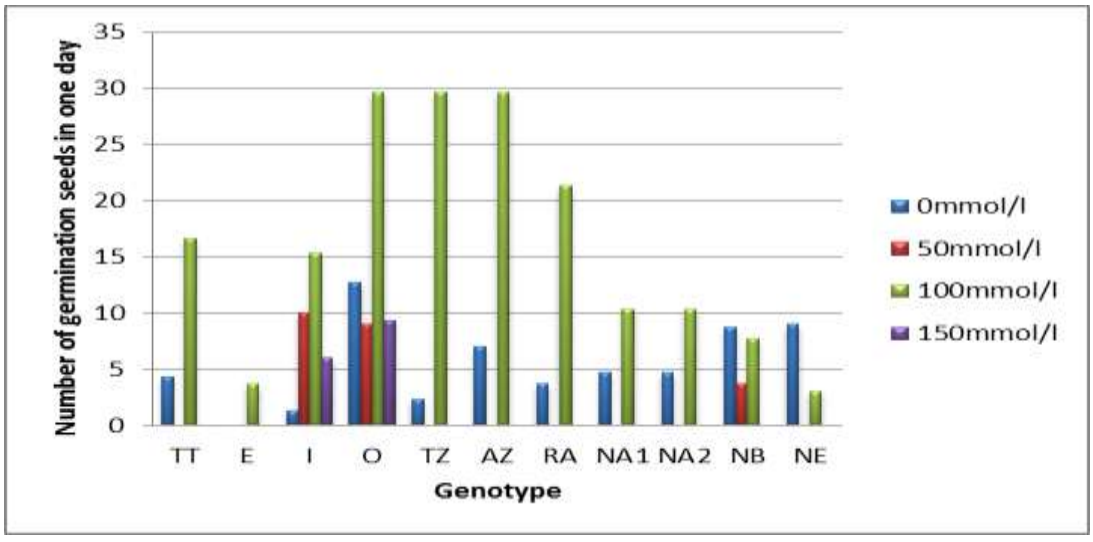



**Figure 24 :** The number of germinated seeds is counted after 24 hours.

According to this figure the varieties of (O), (AZ), (TZ), (RA) is the first varieties which germinated after 24 h and by high germination (all the seeds are germinated) (100mmol/l) but in 150mmo/l the only genotypes who germinated were (O) and (I). While (TT), (TZ), (AZ), (RA) did not germinated in the concentration of 50mmol/l and 150mmol/l.

## 1.3. The germination kinetics:

**Table 8 :** Means of germination kinetics of genotypes in deferent concentration.

| 0mmol//L | | | |
|---|---|---|---|
| | DAY1 | DAY3 | DAY5 |
| **TT** | 4, 33 | 15, 67 | 21, 33 |
| **E** | 0, 00 | 9, 33 | 10, 33 |
| **I** | 1, 33 | 30, 00 | 30, 00 |
| **O** | 12, 67 | 30, 00 | 30, 00 |
| **TZ** | 2, 33 | 30, 00 | 30, 00 |
| **AZ** | 7, 00 | 30, 00 | 30, 00 |
| **RA** | 3, 67 | 26, 00 | 27, 00 |
| **NA1** | 4, 67 | 9, 67 | 27, 33 |
| **NA2** | 4, 67 | 19, 33 | 15, 67 |
| **NB** | 8, 67 | 14, 33 | 18, 33 |
| **NE** | 9, 00 | 1, 33 | 1, 67 |

| 50 mmol//L | | | |
|---|---|---|---|
| | DAY1 | DAY3 | DAY5 |
| **TT** | 0, 00 | 12, 00 | 21, 33 |
| **E** | 0, 00 | 11, 00 | 10, 33 |
| **I** | 10, 00 | 29, 67 | 30, 00 |
| **O** | 9, 00 | 30, 00 | 30, 00 |
| **TZ** | 0, 00 | 18, 00 | 30, 00 |
| **AZ** | 0, 00 | 23, 67 | 30, 00 |
| **RA** | 0, 00 | 16, 67 | 27, 00 |
| **NA1** | 0, 00 | 10, 67 | 27, 33 |
| **NA2** | 0, 00 | 15, 00 | 15, 67 |
| **NB** | 3, 67 | 12, 33 | 18, 33 |
| **NE** | 0, 00 | 1, 00 | 1, 67 |

| 100 mmol//L | | | |
|---|---|---|---|
| | DAY1 | DAY3 | DAY5 |
| **TT** | 16, 67 | 0, 00 | 21, 33 |
| **E** | 3, 67 | 0, 00 | 10, 33 |
| **I** | 15, 33 | 5, 676 | 30, 00 |
| **O** | 29, 67 | 10, 00 | 30, 00 |
| **TZ** | 29, 67 | 13, 33 | 30, 00 |
| **AZ** | 29, 67 | 5, 33 | 30, 00 |
| **RA** | 21, 33 | 0, 00 | 27, 00 |
| **NA1** | 10, 33 | 0, 00 | 27, 33 |
| **NA2** | 10, 33 | 0, 00 | 15, 67 |
| **NB** | 7, 67 | 0, 00 | 18, 33 |
| **NE** | 3, 00 | 2, 33 | 1, 67 |

| 150mmol/L | | | |
|---|---|---|---|
| | DAY1 | DAY3 | DAY5 |
| **TT** | 0, 00 | 1, 33 | 21, 33 |
| **E** | 0, 00 | 1, 00 | 10, 33 |
| **I** | 6, 00 | 15, 33 | 30, 00 |
| **O** | 9, 63 | 12, 33 | 30, 00 |
| **TZ** | 0, 00 | 19, 33 | 30, 00 |
| **AZ** | 0, 00 | 17, 33 | 30, 00 |
| **RA** | 0, 00 | 1, 00 | 27, 00 |
| **NA1** | 0, 00 | 1, 00 | 27, 33 |
| **NA2** | 0, 00 | 0, 00 | 15, 67 |
| **NB** | 0, 00 | 9, 67 | 18, 33 |
| **NE** | 0, 00 | 2, 33 | 1, 67 |

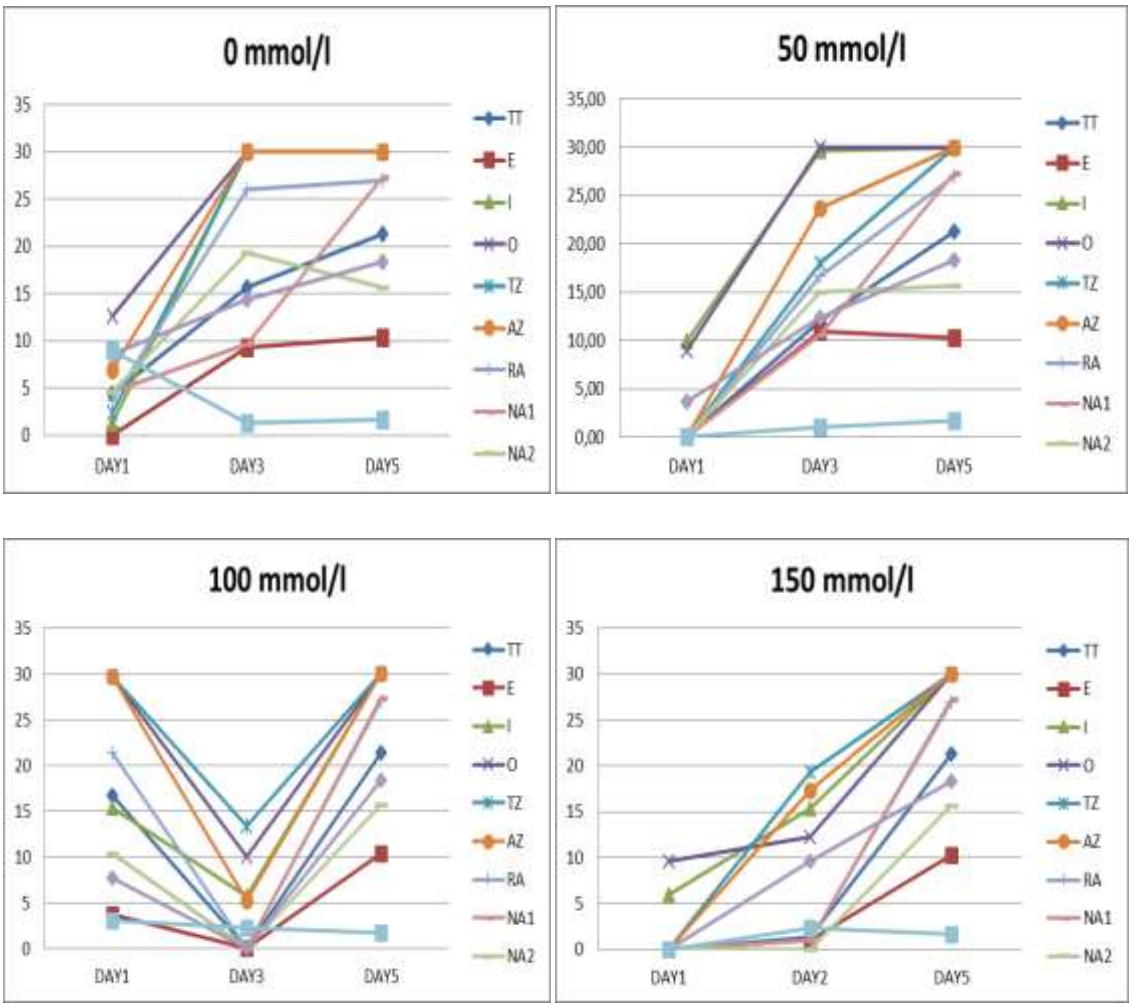



**Figure 25 :** Effects of different salt concentrations on the kinetics of germination of 11 cowpea genotype study for 5 days.

The curves show a diminution of the germination kinetics independing on the increase in salinity, which varies distinctly with the species and the treatment.

In the absence of salt, the seeds germinate after one day, all genotypes were average in all concentrations of NaCl, except for (O), (TZ) And (AZ) were increased in the concentration of 100mmol/l.

In the 3th day the concentration was 50 and 100mmol/l the genotypes (I), (O) and (AZ) had the highest germination kinetics. Only in the genotype (RA), the kinetic was decreasing and for all genotypes in the concentration of 100mmol/l. for the last day the genotypes (I), (O), (TZ) and (AZ) had the highly kinetics and (RA) was very low.

## 1.4. Vigor index:

**Table 9 :** Means of vigor index of genotypes of cowpea.

| | Day 1 | | | |
|---|---|---|---|---|
| | S0 | S1 | S2 | S3 |
| **TT** | 26, 94 | 0, 00 | 64, 44 | 0, 00 |
| **E** | 0, 00 | 0, 00 | 2, 93 | 0, 00 |
| **I** | 14, 70 | 57, 99 | 54, 59 | 10, 00 |
| **O** | 262, 67 | 900 | 75, 164 | 16, 79 |
| **TZ** | 37, 71 | 0, 00 | 75, 146 | 0, 00 |
| **AZ** | 111, 05 | 0, 00 | 85, 05 | 0, 00 |
| **RA** | 40, 44 | 0, 00 | 103, 8 | 0, 00 |
| **NA1** | 31, 79 | 0, 00 | 25, 47 | 0, 00 |
| **NA2** | 44, 19 | 0, 00 | 46, 13 | 0, 00 |
| **NB** | 93, 63 | 20, 3 | 26, 07 | 0, 00 |
| **NE** | 48, 00 | 0, 00 | 0, 00 | 0, 00 |

| | Day 3 | | | |
|---|---|---|---|---|
| | S0 | S1 | S2 | S3 |
| **TT** | 71, 06 | 49, 6 | 0, 00 | 3, 54 |
| **E** | 46, 02 | 63, 05 | 0, 00 | 0, 00 |
| **I** | 308, 65 | 114, 07 | 20, 03 | 15, 55 |
| **O** | 359, 65 | 238 | 25, 33 | 54, 00 |
| **TZ** | 448, 23 | 196, 8 | 33, 76 | 37, 36 |
| **AZ** | 364, 9 | 157, 8 | 15, 22 | 31, 19 |
| **RA** | 244, 13 | 80, 00 | 0, 00 | 0, 00 |
| **NA1** | 42, 53 | 34, 83 | 0, 00 | 0, 00 |
| **NA2** | 138, 76 | 86, 00 | 0, 00 | 0, 00 |
| **NB** | 61, 10 | 47, 9 | 0, 00 | 13, 47 |
| **NE** | 7, 08 | 0, 00 | 0, 00 | 0, 00 |

| | Day 5 | | | |
|---|---|---|---|---|
| | S0 | S1 | S2 | S3 |
| **TT** | 62, 22 | 38, 56 | 82, 47 | 16 |
| **E** | 148 | 3, 83 | 8, 26 | 0, 00 |
| **I** | 14, 3 | 59, 9 | 71, 86 | 34, 45 |
| **O** | 262, 67 | 102 | 26, 16 | 48, 6 |
| **TZ** | 37, 71 | 131, 2 | 34, 6 | 56, 06 |
| **AZ** | 111, 05 | 42, 22 | 16, 21 | 22, 8 |
| **RA** | 51, 03 | 49, 57 | 0, 00 | 0, 00 |
| **NA1** | 193, 51 | 54, 41 | 0, 00 | 0, 00 |
| **NA2** | 9, 54 | 3, 83 | 0, 00 | 0, 00 |
| **NB** | 136, 82 | 53, 5 | 0, 00 | 16, 73 |
| **NE** | 32, 00 | 0, 00 | 0, 00 | 0, 00 |

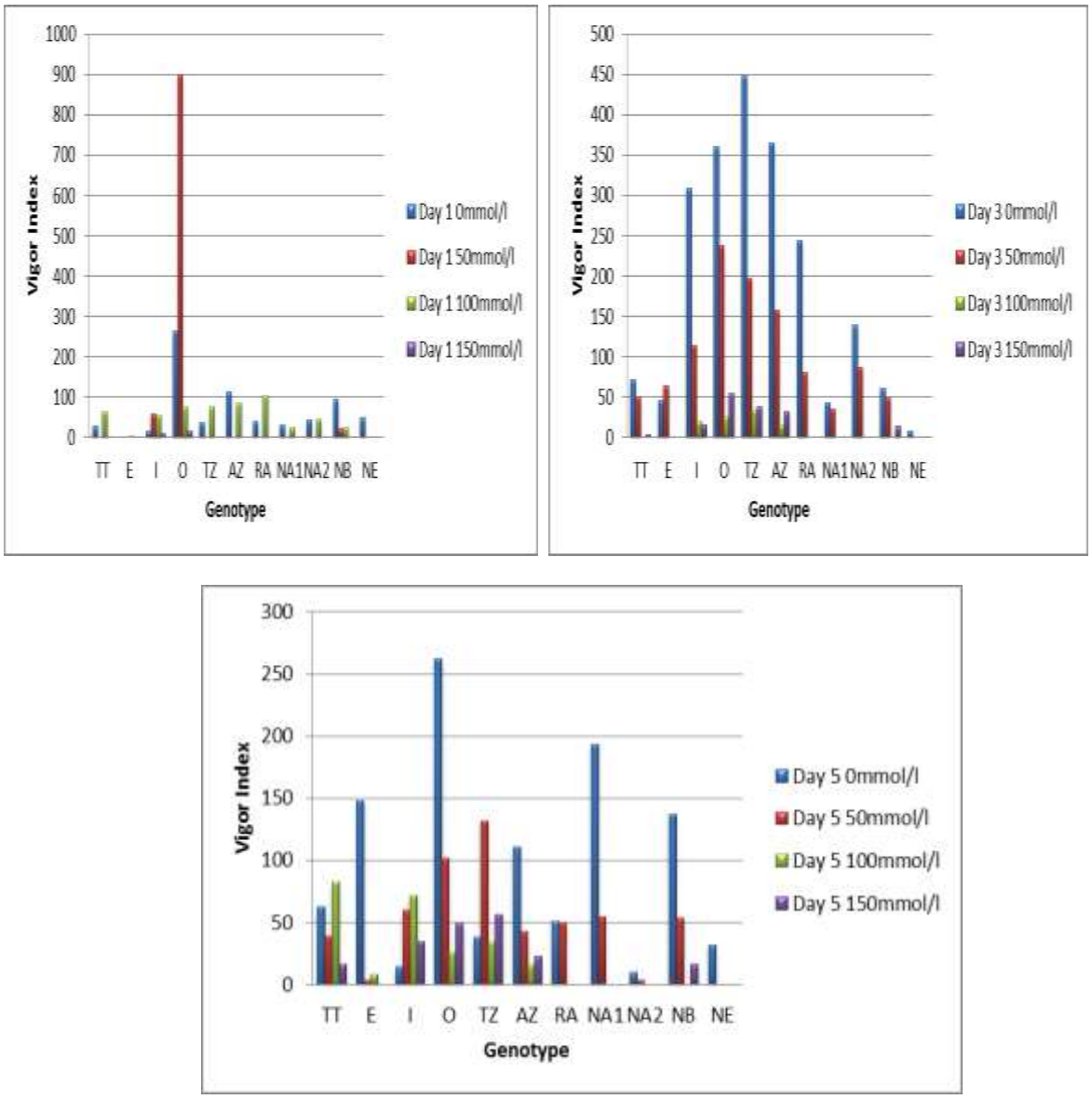



**Figure 26 :** vigor index of different genotype of cowpea.

According to the figure 26. In the day one, we observe that the varieties of (O) have the highest vigor index compared to other varieties for the concentration 50 mmol/l.

In third day, we noted that the vigor index of (O) is decreased in third compared to first day (for the concentration 50). In addition, the other varieties were average in50 mmol/l

While in the concentration 100 and 150 mmol/l were weak for the genotypes (I), (O), (TZ), (NB) and (AZ) but almost none in genotype (TT), (E), (NA1), (RA), (NA2) and (NE).

A noticeable decrease in the vigor index of all varieties in the last day of germination, only the genotypes of (TZ) which have an average vigor index in concentration 50 mmol/l, and for (TT), (I), (O) and ( TZ) there is an increase compared to the province days.

## 1.5. Proline content:

The figure below shows the proline curve for assessing proline content in the cowpea samples.

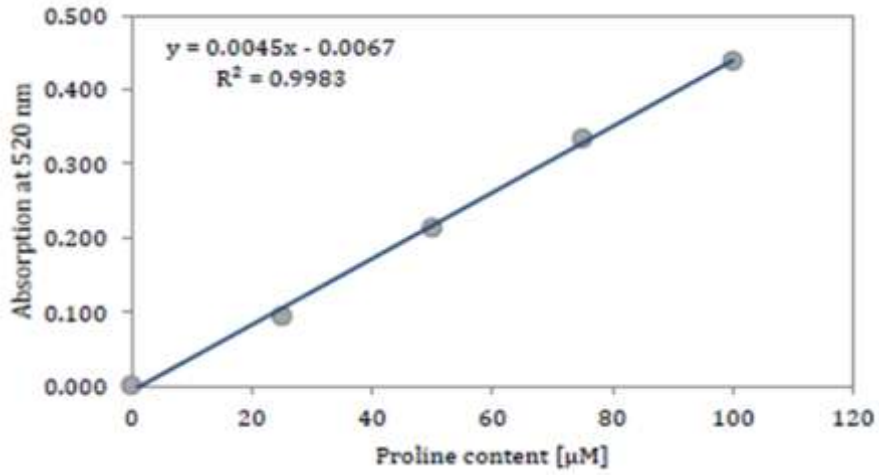



**Figure 27 :** Standard curve for proline content (**Gwendolin and al, 2016).**

## 1.6. Concentration of proline:

**Table 10 :** Means of concentration of proline.

| | S0 | S1 | S2 | S3 |
|---|---|---|---|---|
| **TT** | 55, 19 | 54, 82 | 41, 79 | 58, 45 |
| **E** | 0, 00 | 0, 00 | 0, 13 | 0, 00 |
| **I** | 43, 86 | 52, 67 | 72, 75 | 11, 64 |
| **O** | 45, 86 | 73, 49 | 64, 45 | 35, 27 |
| **TZ** | 34, 53 | 0, 00 | 30, 60 | 25, 12 |
| **AZ** | 55, 93 | 32, 38 | 0, 00 | 0, 25 |
| **RA** | 65, 12 | 32, 38 | 30, 60 | 13, 19 |
| **NA1** | 47, 04 | 13, 19 | 16, 38 | 87, 41 |
| **NA2** | 47, 12 | 43, 34 | 9, 34 | 30, 30 |
| **NB** | 39, 71 | 34, 30 | 28, 97 | 30, 90 |
| **NE** | 0, 00 | 0, 00 | 0, 05 | 0, 04 |

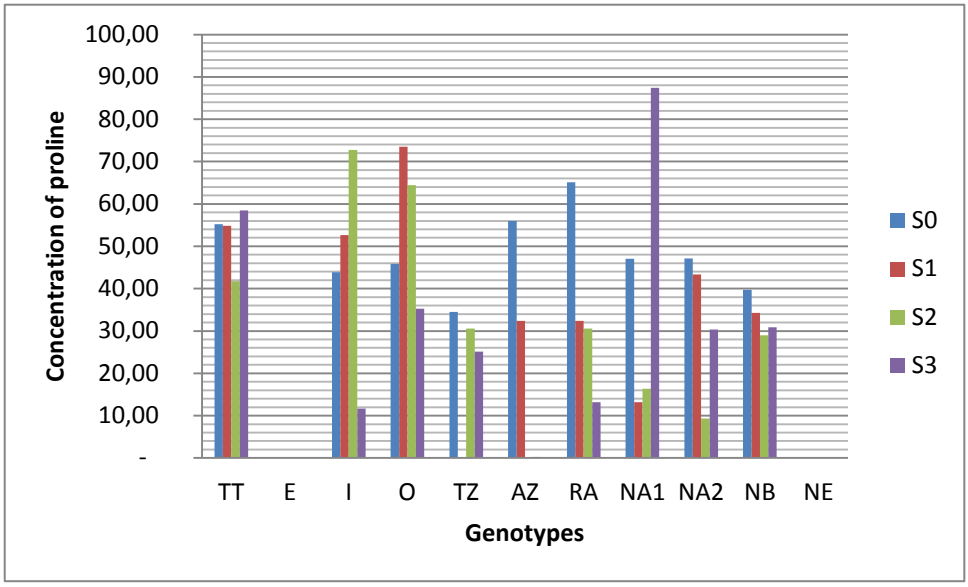



**Figure 28 :** The proline content in 11 genotypes of cowpea.

We observed that between the different genotypes of cowpea for the content of proline in the different levels of NaCl concentration, that:

For (NA1) and (TT) were the one who has the most the greatest value of proline ever produced in concentration of 150 mmol/l.

While (E) and (NE) had the less content proline in all concentrations. Moreover, all the following seeds (NB), (RA), (O), (NA2) and (TZ) were average, and for concentration 50mmol/l and 100mmol/l, the genotypes (O) and (I) had high concentration of proline.

## 1.7. The ANOVA test:

**Table 11 :** Two-way analysis of variance (ANOVA) of cultivar, treatment and their interactions for the parameters under study.

| Source of variation | G day 1 | G Day 2 | G Day 3 | Proline Content |
|---|---|---|---|---|
| | F | F | F | F |
| **Genotypes (G)** | 46, 86** | 99, 10** | 3. 75** | 2, 54* |
| **Traitements (T)** | 402, 59** | 12972** | 41. 84** | 2, 67 |
| **Interaction (GxT)** | 21, 98** | 5, 95** | 2. 2** | 3, 44** |

*, ** Significant at the 0.05 and 0.01 probability level, respectively.

For most analyzed variables, the effects of cultivar, treatment and their interactions were highly significant ($p<0.01$) and for the genotype (G) was significant in ($p<0,05$). The only non-significant value was found in the Treatment (T) of proline content.

For germination all statistical analysis were highly significant ($p<0.01$) salinity has a significant effect on germination between genotypes, all so for interaction, as shown in table 4.

Besides there was a significant effect between salinity effect and treatments except for treatments of proline it is non-significant.

This is confirmed by the analysis of variance which shows a highly significant in the absence of salt (appendix.) where the calculated F 46. 86 is greater than F critical = 2.59 at the threshold of 0.01) hence the test is highly significant.

**2. Discussion:**

Under controlled conditions in distillated water where the osmotic pressure is high, which leads to an optimal germination capacity of the seeds, this confirms the almost general rule on the germination of halophytes (**Belkhodja and bidai, 2004)** and glycophytes (**Meloni and al, 2008)**. In other work has also reported that the seeds of most species reach their maximum germination in distilled water (**Khan and Gulzak, 2003; Naidoo and Keit, 2006; Wei and al, 2008).**

With vigor index, which is defined as the level of activity and performance of the seeds during germination whereas the ability of seeds to germinate was high in varieties (O) and (I), which is explained by the beginning of the division of the meristemtic cells of the root, which leads to its growth, and a delayed germination for others varieties (NE), (NA1), (NA2) and (E) back to that there is a sensitive to salt stress. These results are in accordance with the research of several authors including **Dantas and al, (2005). Gogile and al, (2013), Mshembula and al, (2015).** On cowpea and in several species of legumes by **Okçu and al, (2005)**, among chickpea cultivars, and different bean varieties (**Cokkizgin, 2012).** Other forage legumes (**Wu and al, 2011**). These authors have shown that the salt stress affect the germination rate and caused an reduction significant in germination early in the sensitive genotypes and according to (**Spears and al, 2002).** Vigor tests have proven to be more useful as predictors of field emergence than the standard germination test. When planted in fields with stressed environmental conditions, a high vigor seed lot can withstand the stress during germination and early seedling development longer than a low vigor seed lot.

After the analyzes of the kinetic test of the seeds. Which is expressed as the cumulative average of seeds germinated on different days and at different levels of salt concentration. Demonstrated by **Hadjlaoui and al, 2007**. There is a delay in seed germination at all stresses for the genotype (RA) as well as the decrease in the average daily germination of all genotypes at NaCl concentration100 mmol/l is explained by the time required for the seeds to set up mechanisms that allow them to adjust their internal osmotic pressure (**Judy and Al, 2010).**

Proline is an amino acid when its concentration in the plant is higher than normal, which is indicating of stress, whereas it was high in (NA1), (TZ), (O), (NA2) and (TT) compared to sensitive seeds like (E) and (NE). Our results agree with those of Blkodja, 1996. The proline concentration proved that the salt stress effects on their production and it has been proved by joshi, 1984.

# *Conclusion and Perspective*

**Conclusion and Perspective:**

This work aimed to study the effects of salinity on the germination and synthesis of proline in *Vigna uniguiculata*. (L.) Walp. , 1843.

Our result showed that: The germination ability of genotype Ilizi and El Oued were the highest under salt stress; therefore, they are considered the best genotypes for saline soils, while the other genotypes could be sowing under normal conditions. For the foreign genotypes, the most tolerant one was Niébé d'Asie, this genotypes is interesting for cross program with the susceptible landraces genotypes.

The proline produced corresponds to its high value for the Néibé d'asie and Timimoun genotypes, and is an indicator of resistance to salt stress for those genotypes.

All the results obtained constitute only a first step in the search for the tolerance of *Vigna uniguiculata* to face of salinity as well as its nutritional, economic and agronomic interest. For this reason, it would be necessary to:

This type of been in not widely known in Algeria, despite its importance and therefore we are trying in the future to plant it in the largest possible number of wilaya so that it becomes of high consumption and production through the methods of local population. In view of salinity situation in Algeria, we should plant the varieties Ilizi and El Oued for their resistance in arid and saline area. In the other hand we could improve the tolerate varieties El Oued and Iliziby crossing between (Ilizi and Néibé d'Asie) or (El Oued and Néibé d'Asie) to obtain a good variety that have the character of resistance and good yield.

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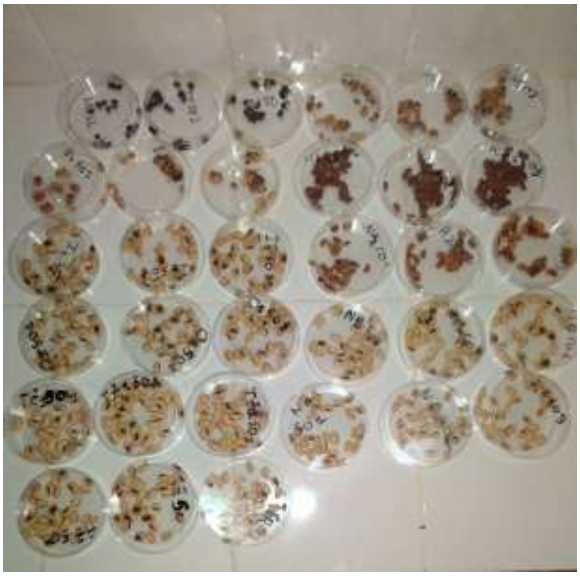
# *Appendices*

- **Germination in Petri dishes :**



- **sowing seeds in pots :**

- **Traitement with NaCl :**


- **Extraction of proline:**

# Theme

## SALINITY EFFECT ON GERMINATION AND PROLINE SYNTHESIS OF THE LEGUMINOUS SPECIES *Vigna unicuiculata QUERCUS sp*

**Presented by:**

- **DJAOUMBI Nourhane**
- **ZEGAD Amel**

**Abstract**

The aim of this work is to study the effect of salt stress at the germination and seedling stage of 11 varieties of cowpea (*Vigna unguiculata subsp. unguiculata* (*L*) Walp), cultivated and marketed in Algeria. The study was carried out in the science and materials laboratory of the university center of Abd Al Hafid Bousouf of Mila, in a favorable growing condition (ambient humidity, light, temperature 25 C° (The seeds were germinated. In Petri dishes containing increasing concentrations of NaCl (0mmol/l, 50mmol/l, 100mmol/l, and 150mmol/l). The germination constants were calculated (Index vigor, Kinetics, Germination rate, Precocity of germination, the proline content). In a second part for the determination of the proline the seeds were placed in a 15/10 cm pot with black peat until the seedling stage, the salt treatments were applied with same way for germination. The results show that salt has a negative effect on the germination rate and the increase of proline levels in some genotypes (O) and (I). However, this effect varies according to the variety and the intensity of the seed stress.

**Key words:** cowpea, salt stress, germination, NaCl, proline.

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