



## Study of Some elements of biology of Wild Jujube «Ziziphus lotus (L.) Desf.

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### Abstract

This study showed that *Ziziphus lotus* (L.) reproduces vegetatively mainly through horizontal roots and root crown located superficially in the soil. Other modes of dissemination by seedlings from root crown, fragments of root crown, branch cuttings and seeds are possible if soil moisture and the temperature are favorable. The stone is a major obstacle to seed germination. The optimum temperature for germination of jujube seeds is 35 ° C. Increasing the temperature to 40 °C severely affects their germination and viability. The application of gibberellins hormone enhances the effect of temperature on seed germination. This implies the existence of two types of seeds dormancy: physiological and physical. The burial of seeds to a depth of 3cm significantly raised their germination kinetics and reduced their final rates. While seeds burial to 6 cm prevents their emergence. The cutting the roots and root crown and their burial in the ground at depths greater than 10 cm stopped their regeneration. After emergence, the seedlings pass through six stages of development and they acquire the status of perennials.

**Keywords:** *Ziziphus lotus* (L.) Desf, biology, vegetative regeneration, germination, burial.

### Introduction

In Morocco, the wild jujube (*Ziziphus lotus* (L.) Desf.) Commonly called Sedra is a species found in many habitats of arid and semi-arid regions. It behaves as a weed in many crops, including winter and spring cereals, food legumes and vegetables and invades rangelands. In addition to the reduction of the rental and resale value of infested land, this shrub affects directly the production of different crops (Rsaissi et al., 2012). Also, the presence of clumps of jujube in constitutes a refuge for many pests or/ and their host (weeds, rodents, shellfish, Spanish sparrows, insect pests and bacteria (Rsaissi et al., 2012). The biological study and population dynamics of jujube under different cultural practices is a very useful basis for understanding the evolution of its infestation of cultivated fields and development to adopt integrated control against this shrub in croplands. In this current study, we sought to determine the following: regeneration, stages of development and acquisition of the status of perennial of this wild jujube.

### Materials and Methods

#### Vegetative Regeneration

To highlight the regenerative capacity of the jujube, we conducted a series of experiments under laboratory and in the natural environment. These experiments were completed by field observations and surveys- questionnaire of farmers.

#### Origin and Sample Collection

Samples of different plant organs were taken from a large clump of jujube infesting a field with sandy loam soil, located in Sidi Aydi (province of Settati). Retrieved organs are horizontal roots, vertical roots, crown roots and stems. These samples were stored until planting in plastic bags filled with the same soil from which they were taken.

#### Regeneration of Different Jujube Organs

Sixteen samples of collected organs of the plant were planted separately in early December in plastic pots containing fine soil taken from the field of their origin. A Randomized Complete Block Design (RCBD) with four replications was used. We considered four pots (samples) as an experimental unit. The pots were irrigated to ensure adequate soil moisture during the experiment period (December to July). The rate of regeneration of various organs planted was calculated separately as a percentage of vegetative cover of each batch of these organs.

#### Effect of Planting Depth and Size of Fragments on Regeneration

The different organs were cut in order to have the following:

- Length of root fragments (vertical and horizontal): 16 cm, 8 cm and 4 cm;
- Length of crown roots fragments: crown roots entire, half and cut into three.

Those organs were planted separately and directly into the soil in pits at different depths (1.5 cm, 5, 10, 20 and 30cm) filled with fine soil taken from the field of their origin. The experimental design adopted was a split-plot design with four replications. The observations were carried on vegetative recovery of each organ planted for each burial depth. The results are expressed as % of regeneration.

#### Seed Germination

*Origin and sample collection*

Fruit samples of two lots of different origins (El Brouj and Rhamna) were used for this study. Thus, we conducted a series of experiments in the laboratory and in the natural environment.

### **Laboratory Experiments**

Germination tests were carried out in Petri dishes (90 mm) on filter paper Whatman No 1. These papers were moistened with 4 ml of distilled water on a regular basis until the end of testing. To prevent fungi attacks, grains jujubes were treated by immersion in a solution of Diféconazol (3mg/l). Three replicates were used at a proportion of 25 grains per Petri dish. Petri dishes were placed in an oven for incubation. The purpose of these germination tests is to measure seed viability (after removal of seed coats) and to explore eventual phenomenon of dormancy, especially embryo dormancy. For this purpose two types of experiments were carried out as follows:

**First experiment:** Its objectives were to determine the specific needs of temperatures for germination and eventual dormancy of jujube grains. For this, four constant temperatures were used: 20, 25, 30 and 40 ° C. At the end of germination test, hard seeds that remained or absorbed water but remaining closed and undamaged are probably dormant.

To identify viable grains but dormant, we used a tetrazolium test according to the procedure below (Rao and al., 2006):

- Preparation of the staining solution to Tetrazolium: we did dissolve 0.5 g of tetrazolium ( $C_{40}H_{32}N_8O_2 - C_{12}$ ) in 100 ml of distilled water.

- Coloration: under the binocular microscope, we cut in half lengthwise grain through the embryo with a razor blade. Then we put half of each grain (the other half is discarded) in the staining solution in a Petri dish. The Petri dish was placed in an incubator for 6 hours at temperature of 30 °C.

- Wash: After staining, we washed the grains with distilled water to remove excess water coloring.

- Observation under a binocular microscope: we evaluated the grains to their mode and degree of coloration. Viable tissue is colored bright red. Very dark pink and red spots indicate dead tissue. We classified the grains into two categories: grains whose embryo is colorful are viable (dormant) and grains that the embryo is not at all colorful are not viable (dead).

**Second experiment:** Its objective was to lift the seed dormancy. We used gibberellic acid treatment at a concentration of 100 ppm combined with three constant temperatures: 25, 30 and 35 °C.

### **Natural Environment Experiments**

The germination experiments were conducted in plastic pots on samples of jujube fruit. The pots were filled with fine sandy loam soil. Three replicates were used at 5 pots per repetition. In each pot, we planted two units of seeds. The experiments focused on the study of the following parameters:

**First experiment:** Its objective was to lift dormancy related to seed coat by using seed treatments prior to germination:

- \* Paring fruit;
- \* Mechanical scarification by creating cracks in the stones (peeled fruit) using a hammer;
- \* Extraction of grains from stones.

**Second experiment:** Its objective was to study the effect of planting depth on the germination of seeds (grains and stones) of jujube. Thus, we used three levels of depth: 1.5, 3 and 6cm.

### **Stages of Development and Acquisition of Perennial Status**

Stages of developing jujube seedlings were determined on the basis of monitoring and observation of a sample of 10 seedlings obtained from experiments in the natural environment. Thus, we noted the date of emergence, the appearance of each leaf in order up to branching. The average time of each stage (ATS) was calculated using the formula adopted by the Lonchamp and Boulet (1989):

$$ATS_i = \frac{\sum_{j=1}^n n_i \times j_i}{N} \quad \text{with} \quad \begin{array}{l} n_i = \text{number of seedling at stage } i \\ j_i = \text{number of days between two consecutive stages} \\ N = \text{total number of seedlings} \end{array}$$

To determine the stage at which the seedling jujube acquired vivacious status, three seedlings of each developmental stage were cut at ground level and monitoring for eventual regeneration.

### **Statistical Analysis**

The results obtained were subjected to analysis of variance after undergoing angular transformations when needed. The software used is STATISTX. A probability of 5% was retained to accept the null hypothesis. In case of significant differences, separation of means was made with Tukey test (Gomez and Gomez, 1984).

## **Results and Discussion**

### **Regeneration**

#### **Vegetative Regeneration**

The results presented in Table 1 and Figure 1 show that the jujube can regenerate from different organs studied. The regeneration of fragments horizontal roots is the most important (90 %). Indeed, these roots have tracer ramifications well equipped of buds capable of emitting ratoon allowing the expansion of the plant if they find moisture they need to grow. While fragments of vertical roots have a regeneration rate (54%) of half less then this rate .After the horizontal roots comes the crown roots with a regeneration rate of 71%. Then, seedlings separated from the mother plant with average regeneration rate of 50%. Statistically, the three types of organs have a different regeneration rate. The pieces of crown roots and branch cuttings produced only 29 and 17% of vegetative regrowth, respectively. The vegetative

awakening took place from February due the increase in temperatures. Stem cuttings and fragments of horizontal roots are the first who developed vegetative buds.

Table 1: regeneration rate of different organs of wild jujube.

organ types	Regeneration rate (%)
Horizontal roots	90 a
Vertical roots	54 bc
crown roots	71 b
Pieces of crown roots	29 cd
Seedlings separated from the mother plant	50 bc
Stem cuttings	17 d

The numbers followed by the same letter are not significantly different according Tukey (HSD) test ( $P \leq 5\%$ ).

### Effect of Organ Size and Depth of Burial on Regeneration

Figures 2, 3 and 4 show a significant decrease in the percentage of regeneration of different organs of jujube depending on the depth of burial in the soil. In addition, the sectioning of these organs into small pieces affected their regeneration rate.

For horizontal roots (Fig. 2), this percentage increased from 50, 60 and 20 at the burial depth 1.5 cm to 80, 60 and 20 at the burial depth of 5 cm and then dropped to 50, 40 and 0 at the burial depth 10 cm, respectively, for the fragments of 16, 8 and 4 cm.

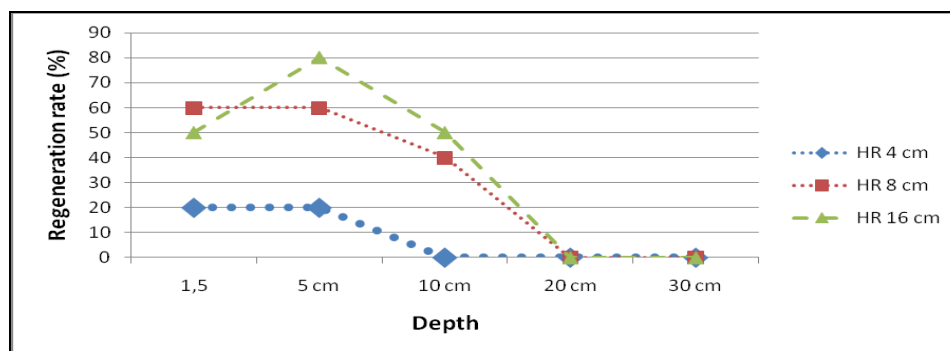


Fig 2: Effect of organ size and depth of burial on regeneration horizontal roots of jujube.

For vertical roots (Fig. 3), the percentage of regeneration for fragments planted at a depth of 1.5 cm was 40 and 20% and then it increased to 60% for fragments of 16 cm and has remained unchanged for fragments of 8 cm (40%) and 4cm (20%), when all of these fragments are buried to 5 cm. At a burial depth of 10 cm, regeneration rate dropped to 20% for fragments of 16 cm, while the other fragments did not produced the regrowth.

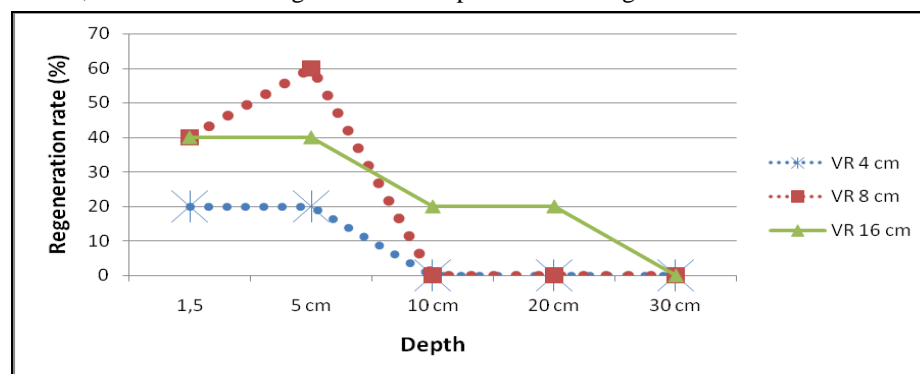


Fig 3: Effect of organ size and depth of burial on regeneration vertical roots of jujube.

As for the crown roots (Fig. 4), at burial depth 1.5cm their regeneration rates were 35, 15 and 0%, respectively, for crown roots entire, crown roots fragmented at half and crown roots fragmented at third. These rates have increased to 50, 20 and 10% when they are planted at 5 cm depth. While only crown roots entire gave regrowth (20%) at 10 cm depth of burial.

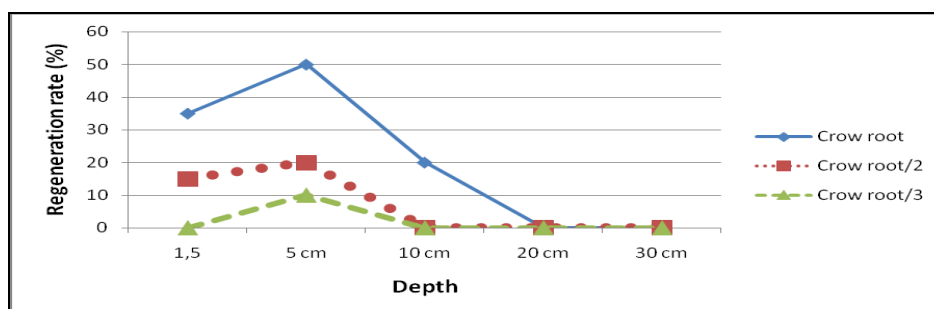


Fig 4: Effect of organ size and depth of burial on regeneration crown roots of jujube.

Indeed, fragmentation of roots (horizontal and vertical) and crown roots and their burial in the ground at depths more than 10 cm made it possible to completely stop their regeneration. The best regeneration rate was obtained for burial depths between 1.5 and 5 cm.

These results show that although of the jujube reproduces mainly through roots and crown roots located superficially and undergoing the different superficial soil tillage (cover crop, hoeing ...) the most dominant in the region. These automatically contribute to its dissemination and its proliferation, especially in irrigated croplands. Fragments of the underground apparatus of this shrub of 4 cm long can regenerate. This high capacity for vegetative regeneration was also discovered in other vivacious plants. So Tahri *et al.* (1988) have shown that fragments of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) to 0.5 cm long are able to emitting viable regrowths and that burial depth has an inverse effect on the regenerative power of this plant and his fragments. Similarly, Omezine and Skiri-Harzalla (2009) reported that rhizomes of *Salpichroa oranifolia* (Lamarck) have a very high capacity for growth and produce many buds and that this regenerative capacity is very important if the rhizomes are cut into small pieces. The longest of these fragments rhizomes give greater biomass than shorter. Also, the buds of these fragments are able to grow at any depth but the emergence and the time required to emerge depend on the amount of carbohydrate contained in the rhizome. To reduce infestation of cultivated fields, it is recommended to use the instruments of labor which at a time permit, either the sectioning of vegetative organs of jujube and their burial deep in the ground, either the extirpation and /or the sectioning of these organs and their exposure to the soil surface during dry periods of the year. So Regher and EL Brahli (1995) reported that extirpation of tufts jujube with Sweep-plow, in June / July and September, have reduced the biomass of the species from 45 to 63% in climatic conditions of the Chaouia region (Morocco).

## Germination

### Results of Laboratory Experimentation

The results presented in figures 5 and 6 shows that the rate of seed viability is between 91 and 96%, respectively, for lot 1 of El Brouj origin and lot 2 of Rhamna origin at temperatures of 20 and 35 °C.

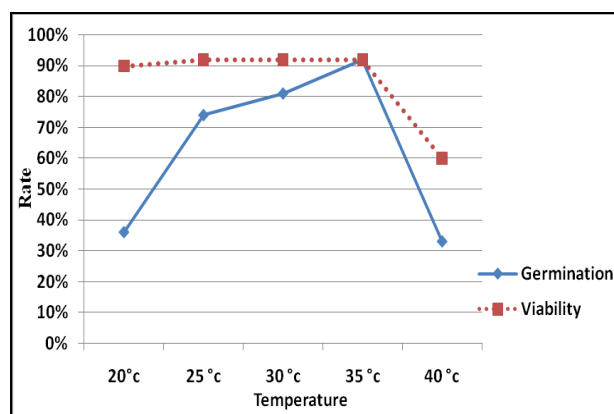


Fig. 5: Effect of temperature on germination and viability of jujube seeds (lot 1)

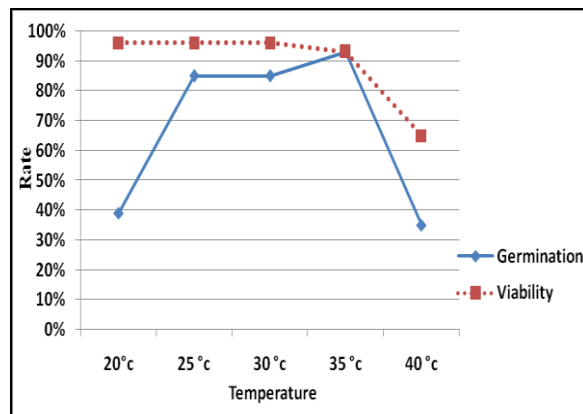


Fig. 6: Effect of temperature on germination and viability of jujube seeds (lot 2)

The effect of temperature on the viability and germination seeds is very marked. The germination rate increases to values greater than 90% at a temperature of 35 °C. Thus, the optimum temperature for germination is 35 °C. Increasing the temperature to 40 °C affected severely the germination and seed viability of the two lots of jujube seeds. At this temperature these rates have respectively dropped to the values of 33-35% and 60-65%. This can be explained, on one hand, by increasing the evaporation of water available, and on the other hand, by a higher increase of oxygen need for the embryo. Consequently, the amount of necessary oxygen decreases and becomes less soluble in water of the envelope (Come and Tissaouit, 1972). This can damage the embryos of some seeds and reduces the performance of germination for other. These characteristics of germination of the seeds of jujube (*Z. lotus*) are similar to those found for other woody species in arid zones: *Ziziphus mauritiana* (Murthy and Reddy, 1989), *Prosopis juliflora* (Gualtieri and Viieira, 1990) and *Acacia segnegal* (Danthu and al., 1992).

Indeed, the temperature affects directly the rate of biochemical reactions and physiological processes which are considered as a phase -activated by an enzyme- essential of these reactions and these can only be achieved that in certain thermal limits (Koller, 1972). The temperature changes can influence a number of process control of seed germination, in particular membrane permeability and activity of cytosolic enzymes (Bewlley and Black, 1994). The temperature range induces the germination process and the emergence differs from one plant to another. It may be shorter or wider depending on the geographical origin of the plant species and the incubation period. For most species, the optimum temperature range for germination and emergence is often high (20-30 °C) (Koller, 1972).

The results of treatment with gibberellic acid (Fig. 7 and 8) combined with different temperatures show that this hormone reinforces the effect of temperature on the germination of two lots of grains of the jujube. This has increased their rate of germination of 16 and 9% at the temperature 25 °C and 12 and 13% at temperature 30 °C, respectively, for lot 1 and lot 2. This presupposes the existence of embryo dormancy for some seeds of this shrub.

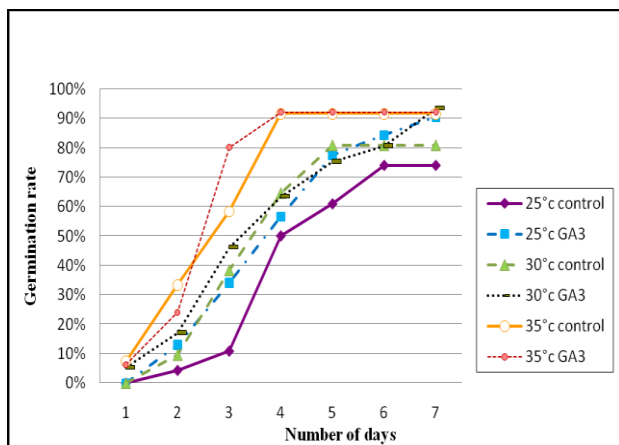


Fig.7: Effect of treatment with gibberellic acid on seed germination of jujube (*Ziziphus lotus* (L) Desf.) (Lot 2.) under different temperatures

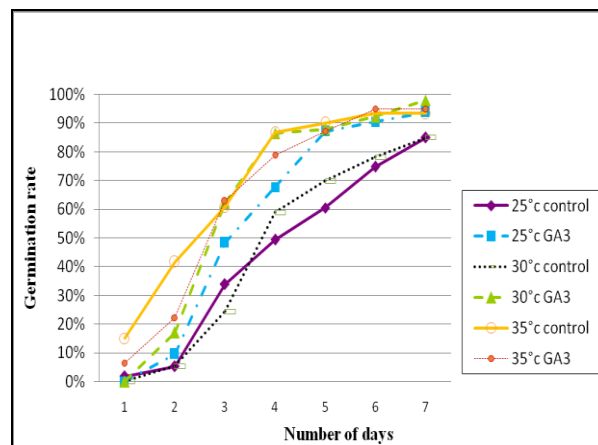


Fig.8: Effect of treatment with gibberellic acid on seed germination of jujube (*Ziziphus lotus* (L) Desf.) (Lot 2.) under different temperatures

### Results of Experiments in the Natural Environment

The results of experiments in the natural environment in Table 2 show that the jujube can regenerate from different types of seed if the adequate soil moisture is ensured for a longer period of up to 7 months after sowing. The regeneration of scarified stones (with a emergence rate to 69%) far exceeds that of fruit entire (drupe), intact stones or grains (seeds) that have emergence rates, respectively, of 51, 22 and 18 %. The seedlings of these different types of seeds were carried out at the beginning of December (period of sowing of winter cereals). The emergence was staggered from February to July with a peak in June (Table 2). The first emergences were observed in February to 58 days after sowing (DAS) for grains (2.2%) and scarified stones (4.4%) and the last in July to 210 DAS for intact stones, scarified stones (2.2%) and entire fruits (4.4%). The average time for the emergence of all seeds is 154 days. It varies in ascending order of 102 for grains to 155 for scarified stones, to 170 for entire fruits and to 176 days for intact stones (Table 3).

Table 2: Date and rates of emergence of different types of the jujube seed

Month	Emergence rate (%)				
	Grains	scarified stones	intact stones	entire fruits	average
February	2.2	4.4	0.0	0.0	1.6
March	8.8	13.2	2.2	4.4	7.1
April	6.6	6.6	6.6	11.1	7.7
May	0.0	11.1	2.2	0.0	3.2
June	0.0	31.1	8.8	31.1	17.7
July	0.0	2.2	2.2	4.4	2.2
Total	17.6 d	68.6 a	22.0 c	51.0 b	38.3

The numbers followed by the same letter are not significantly different according Tukey (HSD) test ( $P \leq 5\%$ ).

Table 3: The average time of the emergence of different types of jujube seed

types of seed	Grains	scarified stones	intact stones	entire fruits	average
The average time of the emergence	102 d	155 c	176 a	170 b	154

The numbers followed by the same letter are not significantly different according Tukey (HSD) test ( $P \leq 5\%$ ).

The emergence kinetics differs markedly from one type of seed to another. It is faster as than the emergence capacity is high (Fig. 9).

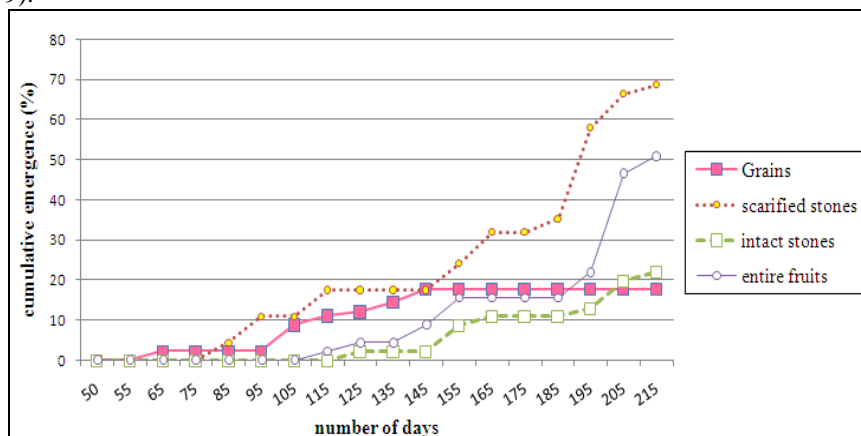


Fig. 9: The emergence kinetics of jujube seed types.



The significant difference in rate, average time and kinetic of the emergence observed between different types of experienced seeds suggests various explanations:

- Grains in direct contact with the sufficient soil moist germinate faster and emerge earlier than other seeds. However, they go moldy and rots;

- The hard envelope of the stone is a major obstacle to germination and emergence by negatively influencing the kinetic and final rate of germination. Baskin et al. (2000) reported that the seeds of the species of the Rhamnaceae family to which the jujube belongs present two types of dormancy: physiological dormancy (due to the low growth potential of the embryo, which may not exceed the mechanical constraint) and a combined dormancy: physical (due to the impermeability of the tegument) and physiological dormancy.

In nature, the abrasion of the endocarp (stones) occurs naturally with time under the action of wind and water, microbial attack and exposure to high and fluctuating temperatures. Physical dormancy of seeds of the genus *Ziziphus* can be broken by two main methods: mechanical and non-mechanical scarification (Pareek, 2001). Mechanical scarification of stones (peeled fruit) has a positive effect on emergence, by favoring the rapid penetration of water and oxygen and their contact with the seed, consequently its germination and the output of radicles and coleoptile. Also, the passage of seeds in the intestines of vertebrates is critical for the dispersal of seeds spread by endozoochory because it improves the germination for some species of *Ziziphus*. Thus, the stones (seeds) of fruits of *Z. mauritiana* consumed by livestock (Grice, 1996), of *Z. lotus* consumed by goats (Inesco and Sauvage, 1965) *Z. mistol* eaten by foxes (Varela and Bucher, 2006) and of *Z. Cinnamomum* consumed by monkeys (Zhang and Wang, 1995), after having passed through the digestive tracts of these animals have a very high germination.

- Fruit pulp surrounding the stone plays sponge role, it absorbs water and maintains a constant humidity that promotes penetration thereafter inside the stone. Similarly, it can serve as a source of organic matter available for seedling growth.

Indeed, the first reaction in the process of germination is imbibition (absorption of water by the seed) which depends on three factors: the composition of the seed, the permeability of the envelope and the availability and mobility of water in its environment (Koller, 1972). Denny (1917) showed that the envelopes of different seed types have different water permeability and he attributed this difference to the structure and composition of the envelope especially these lipid components. The presence of gum in the seed envelopes or the addition of this liquid synthesized industrially improves absorption water (Harper and Beton, 1966). The permeability depends on the volume of collenchyma of the envelope and its expansion and swelling under the pressure of the water penetration into the seed which subsequently promotes the fracture of the envelope, and therefore, the output of the embryo and creating a favorable place for the development of the seedling. Therefore the force of absorption of water that depends on the permeability is considered a very limiting factor that determines the amount of water available for gelatinizing the tissue of the seed during the germination process. The main constituent of the envelope that plays an important role in the penetration of water into the seed are proteins, in addition to other elements of swelling such as gums (Mayer and Poliakoff, 1988). The difference observed in the absorption and the amount of water absorbed process for different seed types is due primarily to the difference in the nature of these proteins (Vertucci and Leopold, 1988). Also, the temperature directly affects the speed of germination and the percentage of emergence by directly acting on biochemical reactions. Because any physiological reaction is an active step enzymes that can only be realized that in a range of well determined temperature (Koller, 1972). To this, we generally consider that any temperature elevation automatically induces the germination process and the emergence. This explains the peak of emergence in June and the low rate of this emergence in February in the case of our experience.

The results of experiments on effect of burial depth on seed emergence, realized from the beginning of February (Fig. 10 and 11), show that the emergence rate of grains and stones severely diminish with depth. The burial of seeds at a depth of 1.5 cm gave the best emergence rate (60 and 33%, respectively for grains and stones). By cons, burial at a depth twice as much (3 cm) allowed significantly slow the speed of emergence and reduce its rate to 72 and 50%, respectively of grains and stones. While jujube seed buried at 6 cm could not emerge from the ground. These results corroborate more or less with the results of the work of Maraghni et al. (2010) who noted a total stop emergence of jujube seed at a depth greater than 4 cm.

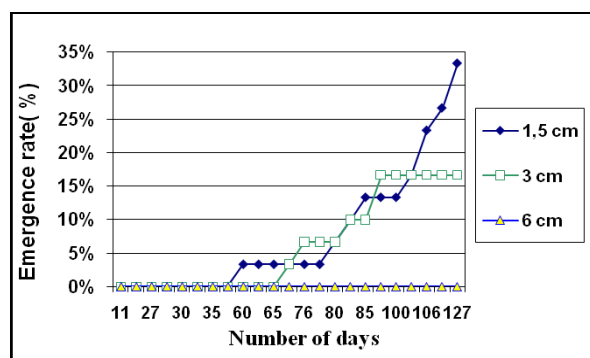


Fig. 10 Effect of sowing depth on emergence of stones (grains with endocarp) of *Z. lotus*

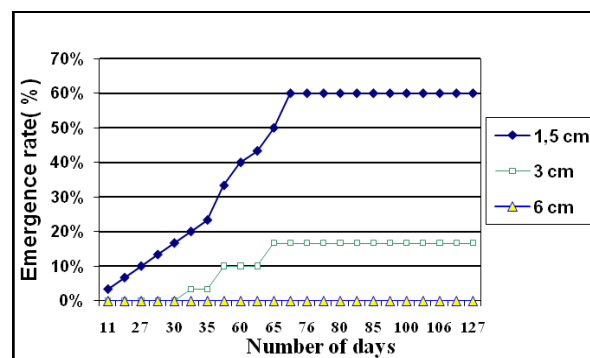


Fig. 11: Effect of sowing depth on emergence of grains of *Z. lotus*

#### Stages of Development and Acquisition of Vivacious Status

The results presented in Table 4 show that the jujube seedlings from different seed types go through six stages of development. After emergence, the average time of each stage of development (ATS) is 14 days for stage of the first leaf, 10 days for stages of the 2nd, 3rd, 4th and 5th leaves and 12 days for the ramification stage. This time varies between

different types of seed tested. This difference may be due to changes in climatic conditions because different seedlings followed have different dates of emergence (between 15 March and 8 May) and to other factors that remaining to be studied. After the sixth stage, the jujube acquires the status of vivacious at an average time of 66 days after emergence.

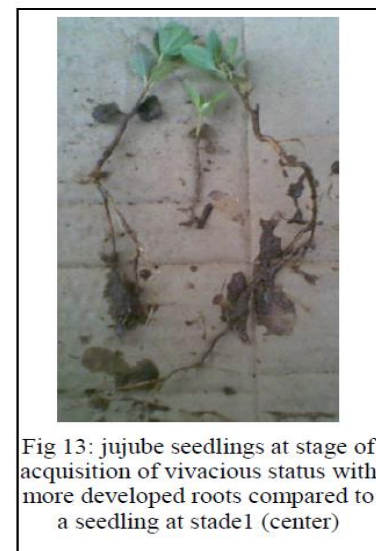
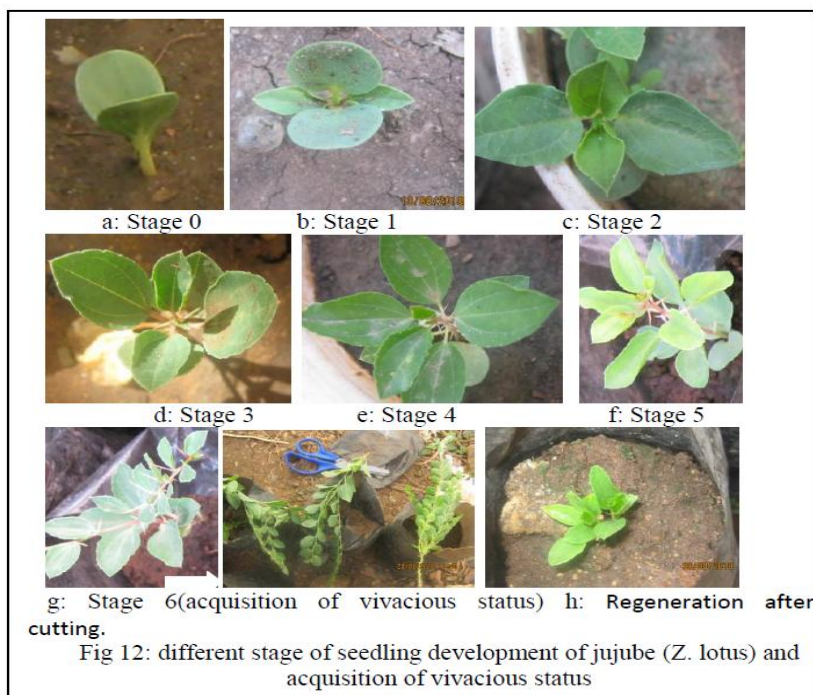
Table 4: Stage of seedling development of jujube et ATS (en nombre de jours)

Stages	Grains	scarified stones	intact stones	entire fruits	average
Stage 0	102	155	176	170	154
Stage 1	13	16	13	14	14
Stage 2	11	10	10	11	10
Stage 3	14	8	8	10	10
Stage 4	9	8	9	12	10
Stage 5	10	10	9	10	10
Stage 6	10	13	13	10	12
Stage 1-Stage 6	67	61	62	67	66

(0) emergence; (1) 1<sup>st</sup> leaves; (2) 2<sup>nd</sup> leaves; (3) 3<sup>rd</sup> leaves; (4) 4<sup>th</sup> leaves; (5) 5<sup>th</sup> leaves; (6) ramification

At the time of germination, the stones broke into two parts. The seed gives a small seedling composed of two equal and opposite cotyledons with long petioles. These cotyledons emerge with the seed coat on their apex (epigeal germination). At the output of seedlings from the soil, in most cases, endocarps of these stones remain in the soil. While in rare cases, they are set on the apex of the cotyledons (Fig. 10). In general, each stone containing two seeds gives a seedling, the other seed remains in its logette and in rare cases this stone gave two seedlings at the same time.

After emergence (Fig. 12), at the stade1, the first two leaves are equal, opposite and petioles. At stage 2, the second leaves are a little alternate but the internodes (starting point petioles) are very short. From this stage, the base of each leaf forms two unequal stipules as very flexible spines. After as and when the internodes between the leaves enlarge and the alternation becomes very visible. In the end, at the stage 6, when the seedling of jujube acquire the status of vivacious plants starts the ramification (curved) of the main stem and leaf petioles become very short. At this stage the roots of these seedlings reach above 15 cm length (Fig. 13) and burrow into the soil to collect water deeply. From the above we can say that this aggressive power of jujube, at the juvenile state, shows that this species is able to invade cropland through some ways that seek its destruction.



## Conclusion

Based on the results of this study, we can conclude that the wild jujube reproduces mainly through horizontal roots and crown roots located superficially in soil and undergo different tillage. This automatically contributes to its dissemination and its proliferation in cultivated land. In addition, other modes of spread by seed, sparkles of crown roots and seedlings separated from the mother plant, pieces of crown roots and stem cuttings are possible if soil conditions are favorable. The viability rate of seed of this species is between 91 and 96%. The temperature directly affects the viability, germination and emergence of these seeds. The fragmentation of roots (horizontal and vertical) into small pieces and crown roots and their burial in the ground at depths greater than 10 cm helped to stopper their regeneration their regeneration. Also, the burial of jujube seed to a depth of 3 cm can significantly slow the kinetics of emergence and reduce their rate of emergence. While burial to 6 cm nullifies their emergence. Seedlings from different seed types (grains, stones, drupes) pass through six developmental stages of the emergence until the 5th pair of leaves. After these stages begin the ramification of the main stem and seedlings acquire the status of perennials.

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