Optimization of carob (*Ceratonia siliqua*) germination in various Moroccan ecotypes

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Abstract- The carob tree is a leguminous plant that is cultivated for multiple purposes. That is, it is grown not only for its production, but also for reforestation in dry and degraded areas threatened by soil erosion and desertification. However, large-scale cultivation of this species is complicated by the difficulty of propagating by cuttings or seeds. In our study, we were interested in propagating carob trees by germination using physical and chemical pretreatments to reduce seed coat hardness. The seeds tested are from his four national ecotypes: Agadir (southwest), Khemissat (northwest), Ouazanne (northwest) and Safi (west). For the scarification we used sulfuric acid, boiling water, pinching the seeds and then soaking them for two days in water, soaking in warm water for ten days, and sowing in Petri dishes. The results obtained showed that the treatment of sulfuric acid and pinching followed by soaking in water for two days are effective since they gave high germination rates (80%) and a short average time of germination (2 - 3 days). Depending on the ecotype, the Agadir region has the best seeds with higher germination compared to other regions tested.

Index Terms- Germination, carob, sulfuric acid, Boiling water, Mechanical Scarification.

I. INTRODUCTION

The carob tree has been grown since antiquity in most countries of the Mediterranean basin, usually in mild and dry places with poor soils. Its value was recognized by the ancient Greeks, who brought it from its native Middle East to Greece and Italy, and by the Arabs, who disseminated it along the North African coast and north into Spain and Portuga [1]. The carob tree (Ceratonia siliqua L.) belongs to the Leguminosae family of the order Rosales. The word carob comes from the Arabic El kharroub. In Morocco the carob tree called kharroub, slaghoua or tikida [2]. The carob tree is particularly abundant in certain areas (Marrakech, El ksiba, Khenifra, Beni Mellal, Meknes, Essaouira, Elhaouz, Kasba Tadla), it offers the means, to the farmers of this region, to put in value lands unusable for all other cultures. These regions could become centers for carob production in Morocco. There is no reason why the carob tree should not succeed in these areas, where we can see some specimens growing without care and giving interesting yields [3]. The carob tree is an economically important multipurpose tree and can also be used for charcoal, wood industry and to prevent soil erosion [4]. Commercial propagation of carob is a major constraint to the cultivation of carob. The plant is propagated commercially by grafting and vegetative propagation by cuttings has not been achieved commercially. Grafting requires generation of seedlings and carob seeds are very recalcitrant and have a physical dormancy [5]. Several attempts at in vitro propagation have also been made in carob at the industrial and/or scientific level [6]. The production of carob (Ceratonia siliqua L.) seedlings is recently encouraged in Morocco due to, among other things, the particular economic interest of its pods, and the mass production of seedlings in nurseries is done mainly by sowing [7]. Indeed, seed germination is difficult without pretreatment [8, 9, 10, 11]. The purpose of this study is to perform chemical and physical scarification pretreatments on carob seeds from four Moroccan regions namely Ouazzane, Khemissat, Safi and Agadir, in order to appreciate the best treatment to reduce the hardness of the seed coatings and to estimate the confined area with a high germination rate.

II. MATERIALS AND METHODS

The present study focused on carob seeds from four Moroccan regions, namely Agadir, Ouazzane, Khémissat and Safi. The germination test was conducted in a greenhouse at Ibn Tofaïl University.

Figure 1: Seeds from the four regions studied.

1. Scarification treatment
   - Controls without treatment
     Sowing seeds from each region without treatment (control).
   - Treatment with sulfuric acid
     Soaking in a beaker containing sulfuric acid (98%) for 20 min.
   - Treatment in boiling water
     Soak the seeds in hot water for 20 minutes.
   - Pinch treatment and two days soaking in water
     The carob seeds were mechanically scarified with a small nail cutter to facilitate their soaking in water for 48 hours.
   - Soak in warm water for ten days
     The seeds are put in glass bottles full of warm water for ten days at room temperature.
   - Processing in petri dishes
     Germination is carried out in Petri dishes with a diameter of 9 cm, with two sheets of absorbent paper inside, and the seeds sprinkled with water.

Figure 2: different treatments used (a: without treatment; b: with sulfuric acid; c: in boiling water; d: Pinch treatment and two days soaking in water; e: Soak in warm water for ten days; f: Processing in petri dishes).

2. Kinetics of germination
   - Seed germination rate (GR)
     The percentage of seeds that have actually germinated.

$$GR(\%) = \frac{SGN}{TNS} \times 100$$

With: NGS = Number of germinated seeds
and TNS = Total number of seeds

- Average germination time (TGA)
  Is calculated from the following formula:

$$TGA = \frac{N1T1 + N2T2 + N3T3 + \cdots . + NnTn}{N1 + N2 + N3 + \cdots . + Nn}$$

N1: Number of germinated seeds at time T1
N2: Number of germinated seeds at time T2
N3: Number of germinated seeds at time T3
Nn: Number of germinated seeds at time Tn

III. RESULTS AND DISCUSSION

1. Seed germination rate (GR)

The germination rate between seeds of the studied ecotypes according to the treatment used is illustrated in Figure 3.

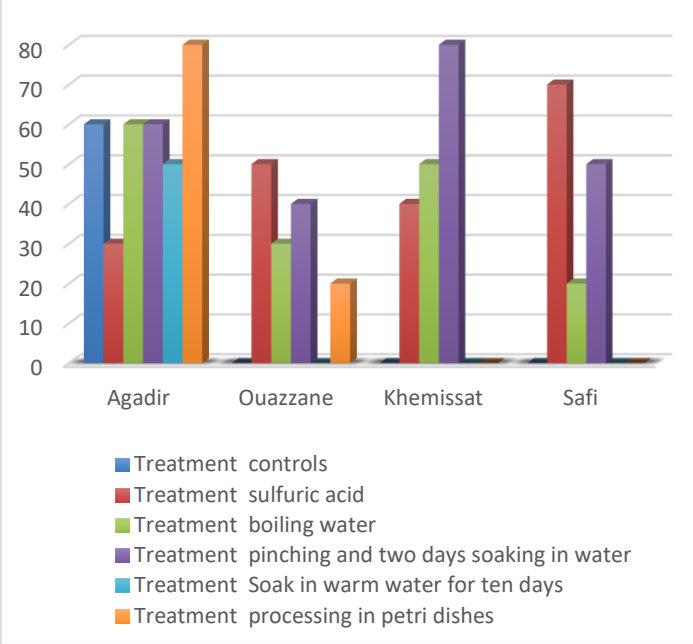

Figure 3: Variation of germination rate according to treatments and ecotypes.

The best average germination rates are noted in the Agadir ecotype with the seedling treatment in petri dishes, also in the Khemissat seeds with a pinching treatment followed by soaking in water for 2 days.
Sulfuric acid treatment and pinching gave significant levels in all seeds tested, and boiling water gave somewhat significant levels.

2. Average germination time (TGA)

The average germination time between seeds of the studied ecotypes according to the treatment used is illustrated in Figure 4.

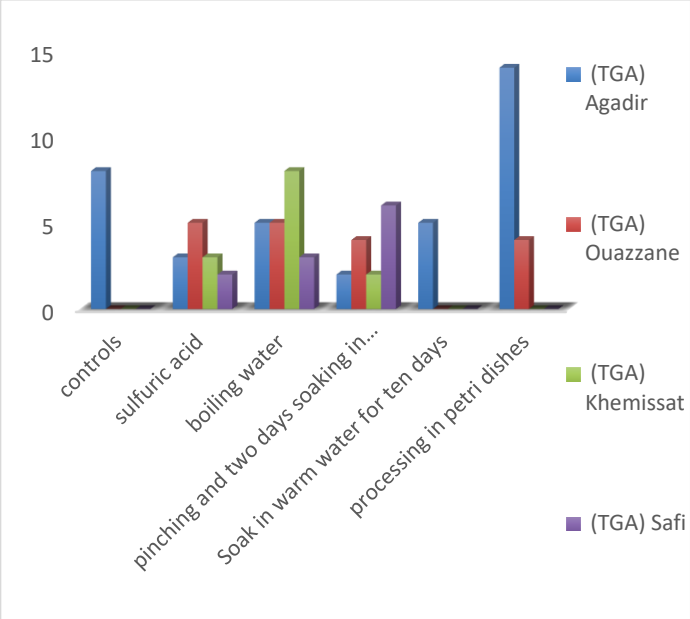

Figure 4: Average germination time for the different ecotypes studied with the pretreatments used.

The nature of the pre-treatment used affects the average time of germination of the seeds. The shortest average time of germination is noted for the pretreatment by pinching and sulfuric acid, it is located from 2 to 5 days for sulfuric acid and 2 to 6 for pinching. For the other treatments, the germination took place only from 5 days, and even it took 14 for the pretreatment in petri dishes.

Figure 5: Results of sowing with the treatment of sulfuric acid.

Figure 6: Results of sowing with the treatment of pinch treatment and two days soaking in water.

- **Comparison between ecotypes**

Concerning the ecotype we note that the seeds of the region of Agadir have an important power of germination, since we obtained rates higher than 50% almost in all the treatments carried out on these seeds.

- **Discussion**

Similar seed dormancy characteristics have been reported in carob by [10], who used various treatments to improve the germination of carob seeds and found the lowest germination rate in control seeds (25%) and the highest (99%) in scarified seeds. Seed dormancy can be broken by mechanical scarification and certain pretreatments such as acid, hot water and dry heat treatments [12]. Similarly, [13] found that the highest germination rate (87%) was found on seeds soaked in concentrated sulfuric acid for 15 minutes, but the shortest average germination time (8 days) was observed with soaking in hot water for 5 minutes. [14] showed that seeds did not germinate in the control group, the highest germination rate for the sulfuric acid treatment was observed in 95% sulfuric acid at 88.90%, and the highest germination rate for the gibberellic acid treatments was observed at 1000 ppm at 28.90%. In addition, the seed of Argan does not present a problem of true dormancy. It is affected by a tegumentary inhibition that could be eliminated by a pre-treatment with warm water or hydrogen peroxide, which facilitates the bursting of the nuts and the access of oxygen to the embryo [15].

IV. CONCLUSION

Germination is the most dynamic stage of the plant growth process, and for the carob tree germination is difficult without scarification while pre-treatment is required to optimize germination rates. It was concluded in this study that sulfuric acid and pinching followed by soaking in water for two days were the most effective pretreatments that gave a high germination rate and a reduced mean germination time. According to the ecotype, seeds from Agadir have a great ability to germinate, they gave the best results for all pretreatments compared to other regions. Farmers and nurserymen who wish to plant carob orchards, especially in dry areas, will be able to use these results to increase their yields. In perspective, we suggest to test the varieties of other wild and cultivated regions, and to study the variation of the duration of the soaking either in sulfuric acid or in warm or boiling water to deduce the optimal time that gives high rates and also the variation of the substrate and also the season to conclude the best conditions to have high germination rates.

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