Effect of substrate, chemical treatment and microclimate on the regeneration of the Argan tree (*Argania spinosa*.L) by greenhouse cuttings.

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Abstract- The objective of this work is to study the effect of three substrates and type of cutting as well as the chemical treatment and the microclimate on the success of cuttings from the Argan tree (*Argania spinosa*.L). The results obtained showed that, depending on the type of substrate, a survival rate of 58.33%, 33.33% and 42.33% respectively for substrate 1 (soil only), substrate 2 (1/2 sand and 1/2 compost) and substrate 3 (1/3 soil + 1/3 compost + 1/3 peat). Regarding the type of cutting, high rates were prevalent in the apical cuttings. For the substrate effect, we obtained a survival rate of 58.33%, 33.33% and 42.33% respectively for substrate 1, substrate 2 and substrate 3. Considering the type of treatment, the development of the buds took 33 days, 37 days and 41 days, respectively for product 1 (Auxin), product 2 (mineral-based) and product 3 (Seaweed extract). As a result, the increase in temperature and humidity, reduced the budburst time by up to two weeks, with the formation of new shoots numbering 4 to 5 per cutting and 15 to 20 new leaves. In our study, we encountered rooting problems, which led us the shoots acquired in the herbaceous grafting technique.

Index Terms- *Argania spinosa*; Apical; Basal; Cutting; Compost; Substrate

I.INTRODUCTION

The Argan tree (*Argania spinosa* L. Skeels) occupies an important place in the Moroccan forest heritage. This agro-sylvo-pastoral species is characterized by an increased adaptation and endurance to face environmental stresses. It occupies about 948,200 hectares [1]. It is the second largest forest species in Morocco after holm oak [2]. The Argan tree is of growing interest not only because of its extremely important role in the fight against erosion and desertification, but especially because of its crucial socio-economic role in traditional rural life [3]. Indeed, its fruits are at the origin of Argan oil, which is the subject of annual commercial transactions on the national and international market, the monetary value of which is constantly increasing.

In Morocco, the Argan tree is one of the main agro-forestry trees because of its characteristics as an endemic tree and the importance of the area it occupies. However, under the pressure of changes linked to anthropometric activities, the fragile balance, previously established between them, the capacity of adaptation and regeneration of the Argan tree and the strong pressure it is undergoing has done irreversible harm to this perennial plant [4]. This species has known over time, significant disturbances related primarily to climate and action essentially antropozoogenic resulting in massive logging and the uncontrolled use of Argan wood for charcoal production. In this way, this plant has been harvested in an unsustainable fashion.

The discovery of charcoal testifies to the persistence of degrading practices in the area of the Argan tree. Demographic pressure, deforestation, uncontrolled urbanization, overexploitation of water resources and pollution combine with global warming to pose serious threats to the durability and sustainability of the Argan forest [5]. These changes result in a decline of complex origins resulting in a degradation and a worrying regression of its surface and genetic resources. In addition, natural regeneration is practically absent in the main distribution area of the species [6]. The objective of this work is to study the effect of three substrates and sampling type (apical or basal cutting) as well as chemical treatment on the success of Argan tree (*Argania spinosa. L*) cuttings under greenhouse conditions.

1.Geographical distribution of the Argan tree

In Morocco, the Argan tree is the second forest species after the holm oak. It covers about 870,000 ha, representing nearly 17% of the country's forest area, with a density ranging from 10 to 50 trees/ha [7].

Overexploitation and abusive logging by humans throughout the last century have led to an estimated annual deforestation of 600 ha [8]. The Argan area is essentially spread over the provinces of Essouira (130,000 ha), Agadir (37,000 ha), Chtouka Ait Baha (90,000 ha), Tiznit (140,000 ha), Taroudant (360,000 ha) and Inzeguane Ait Melloul (13,000 ha) [7]. The main distribution area of the Argan tree is located between 29° and 32° North latitude [9].

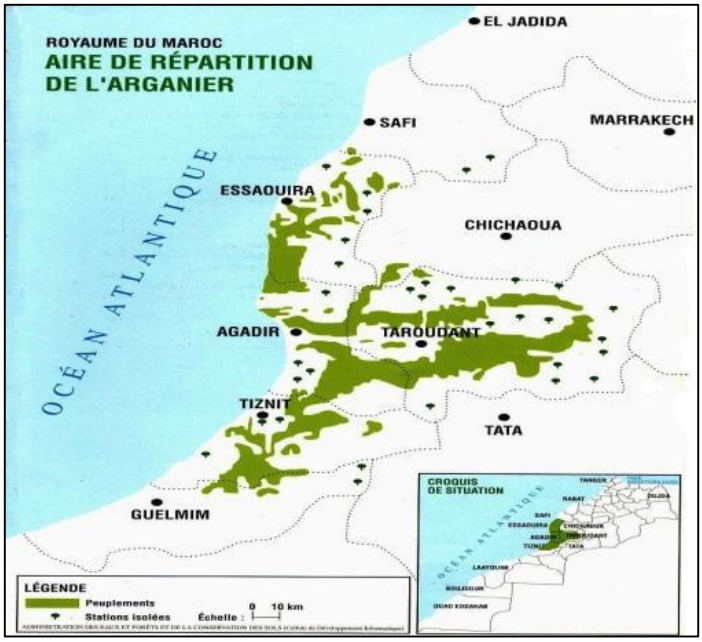

Figure 1: Geographical distribution of the Argan tree in Morocco [10].

2. The multiplication of the Argan tree

The regeneration of the Argan tree in natural forests is currently very weak or even absent because all the nuts are carefully collected for oil extraction. Animals systematically browse young seedlings from seeds that escape harvest. The development of a national strategy for the conservation of this tree is therefore essential. It must first be based on the establishment of a dissuasive and effective protection system to protect the tree from its aggressors.

Intensification of reforestation is another aspect that must be addressed in order to reconstitute lost colonies. However, it should be remembered that propagation (cuttings and transplants) of this species is not easy to achieve and is, at present, a real problem that seriously hinders its spread [11] and [12].

3. Propagation by sexual means

Propagation by seed is the most common way to reproduce forest species. This method, known as sexual propagation, is characterized by a high variability in the progeny and does not allow the conservation of the genetic characteristics of the mother plant [13]. The regeneration of the Argan tree by natural seedlings is possible but exceptional because of the delicate conditions of the germination of the seeds and especially of the survival of the plantlets after germination. Direct seeding, which used to be widely practiced, has been abandoned and is not recommended because of repeated failures and difficulties in germination and development and the risk of destruction by rodents and goats [14] and [15].

According to [15], this approach could lead to satisfactory results if the seedbeds are well prepared.

4. Vegetative propagation

Vegetative propagation by various techniques is an alternative to sexual propagation, as the latter is currently very weak or absent. In contrast to sowing, vegetative propagation does not lead to genetic recombination. The genome of the daughter plant is identical to that of the mother plant. The multiplication of the Argan tree by vegetative way knows, these last years, a notable progress. Several techniques for obtaining Argan plants are currently used, notably herbaceous cuttings, grafting and aerial layering [16]. These propagation techniques are very effective in maintaining desirable traits such as oil content, fodder value or tolerance to abiotic stress [17].

II.MATERIALS AND METHODS

1. Plant material

The genus *Argania* belongs to the phylum of Ebenales and the tropical and subtropical family Sapotaceae, which includes about 600 species, divided into about 50 genera. *Argania spinosa* (L.) Skeels, is the only species representing this genus in Morocco and Algeria [18] and [19]. [19] describes the Argan tree as a third-growth tree, which looks somewhat like an olive tree (Figure 3.B). It is a thorny tree, which can reach 8 to 10 meters high [20] and [21].

Its longevity can reach 150 to 200 years and it is very resistant to drought and heat [22]. The trunk, often short, is made up of several intertwined stems from the welding of very neighbouring shoots or stems from the same core. The dense, rounded crown develops thorny branches on which abundant fruiting bodies flourish (Figure 2).

2. Preparation of substrates and rooting products

To study the effect of the substrate, three types of compositions were chosen:

- **Substrate 1**: Composed only of soil (Maâmora Forest soil, Kenitra);
- **Substrate 2**: Composed of 50% sand and 50% compost;
- **Substrate 3**: Mixture of three compounds distributed as follows;
  - ✓ 1/3 of soil
  - ✓ 1/3 of commercial peat
  - ✓ 1/3 of Compost

The sand used in this study was recovered in the forest of Maamoura. Thus, the compost, was obtained in the urban forest "Saknia" in Kenitra. Morocco.

Figure 2: General aspect of the Argan tree (A), the fruit of the Argan tree (B) [23].

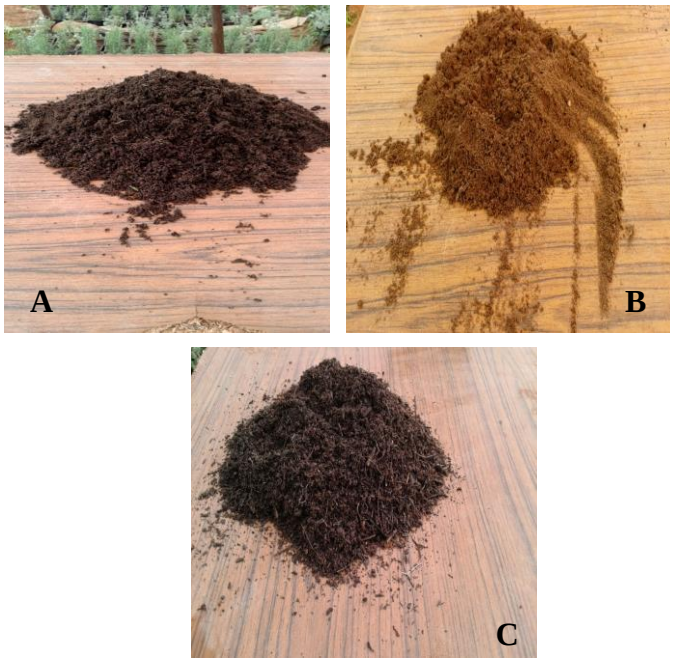

Figure 3: Compositions of the substrates used. (A: peat / B: soil / C: compost).

A step of preparation of the cuttings has been made before the cultivation, this step consists of defoliation of the cuttings, by reducing the number of leaves and the leaf surface, in order to minimize evaporation and avoid the drying of the cuttings. In addition, the apex of each cutting is cut to cancel the apical dominance and stimulate the development of axillary buds and the rooting process. In the second stage, the cuttings are soaked in 3 products (P1, P2, P3) which differ in chemical composition and active ingredient.

The preparation of dipping solution was carried out according to the instructions for each product.

- Product 1: auxin-based.
- Product 2: mineral-based.
- Product 3: seaweed extract based on mineral matter and amino acids.

To realize our tests, we adopted an experimental device (Table 1), which consists of 24 pots for each type of cuttings (apical and basal) or 48 pots for each substrate (S1, S2, S3) or a final number of 144 tests.

Table 1: Experimental device adopted.

Arganisa spinosa.L (144 pots)	S1 (48 pots)	(24) Ap
		(24) Ba
	S2 (48 pots)	(24) Ap
		(24) Ba
	S3 (48 pots)	(24) Ap
		(24) Ba

(Ap: apical / Ba : basal / S : Substrate).

3. Test site and cultivation

We carried out our tests in a plastic tunnel greenhouse, located at the Faculty of Science, Ibn Tofail University in Kenitra (Morocco). To study the effect of temperature and humidity, a mini plastic tunnel greenhouse with a height of 1 meter and a length of 2.5 meters was adopted. The cuttings were grown in honeycomb trays and in closed pots and plastic bags.

III. RESULTS

1. Percentage of successful cuttings.

Figure 4 shows the percentage of success of Argan tree cuttings (Apical, Basal) that have undergone three treatments and grown on three different substrates (sub1, sub2, sub3). For substrate 1 (soil) high percentages (83.33%) were obtained in the apical cuttings treated with product 1 (Auxin), in the same substrate the basal cuttings have lower percentages and vary between 16.66% and 58%.

In substrates 2 and 3 we notice that the percentage of success is almost similar for the two types of cuttings (apical and basal), as well as a similarity between treated and control cuttings (Figure 1).

Statistical analysis by the ANOVA test did not reveal any significant difference between the two types of cuttings and between the different treatments (P>0.05) (Table 2).

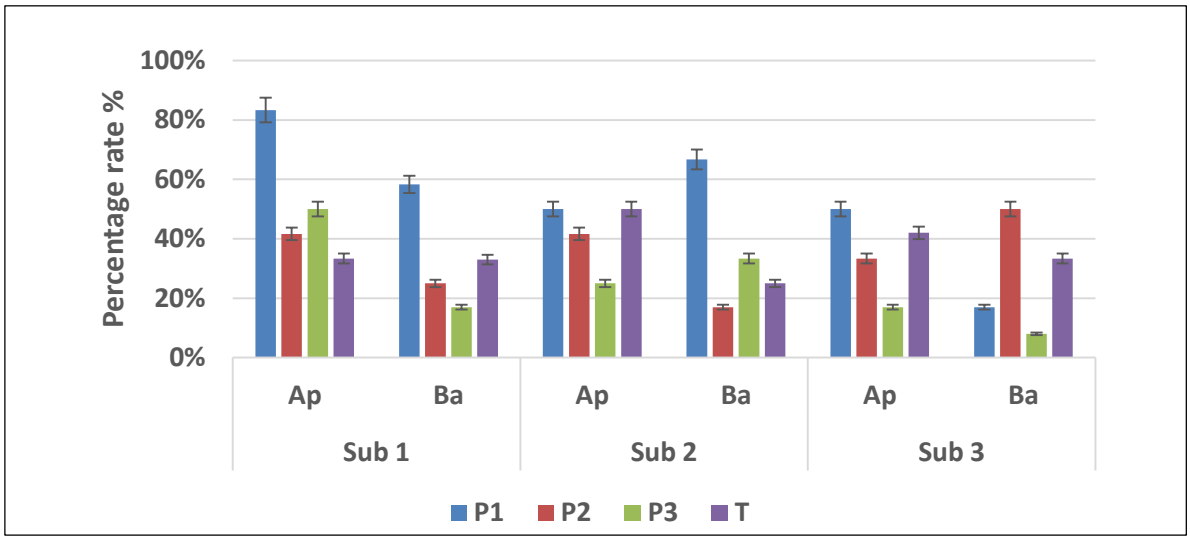

Figure 4: Success rate of argan tree cuttings that have undergone the three treatments in the three substrates. (Sub: substrate / Ap: apical cutting / Ba: basal cutting / P: product / T: control).

Table 2: Statistical analysis by the one-factor ANOVA test on the success rate of argan tree cuttings.

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F	Probability	Critical value for F
Between Groups	0.145	5	0.029	0.890	**0.507**	2.772
Within the groups	0.587	18	0.032			
Total	0.733	23				

2. Aerial part and root system

The success of cuttings, the survival of propagules and the growth after the installation phase, on site of experiment are strongly influenced by the interaction of several parameters including the quality and genetic origin of vegetal material, the rooting substrate, the environmental conditions and the physiological state of the cutting, etc.

In our study, we realized that the cuttings of the Argan tree first developed new shoots and several leaves (figure 5.A) in the first 4 weeks of cultivation. Afterwards all cuttings rotted away and the leaves dried out (figure 5.B).

Figure 5: Example of an Argan tree cutting with new shoots (A) and in a state of dryness of the formed leaves (B).

IV. Discussion

The regeneration of the Argan tree in the natural forest is currently very weak or even absent because all the nuts are carefully collected for the extraction of the oil. Animals systematically graze the young sporophytes developing from the seeds that have not been harvested. Considering all the results we got during this study, it is obvious that germination is the only effective means today for the multiplication of this species. According to [15], this way could lead to satisfactory results to such an extent as the grounds of sowing are well prepared, thus, [24] quoted that in Morocco, the Argan embryonic plants are produced in nurseries from seeds. [25] Also, confirm the results achieved in this study, which led the first work of multiplication of the argan tree by cuttings. These trials are made from herbaceous propagules from adult trees. This technique was conducted under nebulization. The results gained were poo (17% rooting). In the vegetative propagation [17] also mentioned that the genetic variability of the Argan tree is a major constraint to its propagation by cutting. It is proving difficult to develop a single optimal rooting medium for the different genotypes. This type of constraint is common in woody species for which it is often necessary to adapt the nutritional and microclimate conditions according to the requirements of each clone [26] and [27].

Despite the encouraging results we have brought in, the root growth problem is difficult to solve in the Argan tree. In this sense, [28] also proposed that this problem could be attributed to the absence of mycorrhizae (*shrubby endomycorrhizae*) which often help roots to develop better.

There was also significant variation in the percentage of success depending on the type of substrate. [29], Confirm this too, he showed that substrate quality is a very important parameter for the success of the rooting process of cuttings. In addition, the requirements of the species in relation to the different substrates depend on their hydromorphic or xeromorphic character. It would also seem that there is a relationship between the moisture content of the substrate and the cutting. For example, it is known that different substrates affect the water supply of cuttings and have an effect on photosynthesis and stomatal conductance of cuttings [30].

The cuttings will root well in sand-based charcoal potting soil, but in this medium, the risk of drying out is great and it is more prudent to add some peat or vermiculite to the substrate to conserve moisture. However, various mixtures of compost, perlite, peat and perlite, peat and sand, etc. have proven effective. This is why these substrate mixes were chosen for this study [31] and [32].

Given its multiple roles, our Moroccan country has been engaged for over half a century in a major program of reforestation and reforestation of the argan tree [33]. However, these plantations were characterized by a very low success rate due mainly to the use of poor seed sources, overgrazing the lack of a specific technical itinerary for this particular species, the lack of standards and criteria for the morphological and physiological evaluation of the quality of the plants as well [34]. In addition, the heterogeneity of the plant material and the time needed to start production are major obstacles to the development of this crop. The various works on cuttings demonstrated the technical and physiological limits of argan tree cutting. Its success remains dependent on several parameters, thus, even if the cuttings give high rooting results, the plants from these cuttings perish in practice in all cases [13].

V. CONCLUSION

At present, the development of aromatic and medicinal plants through their domestication or cultivation is proving to be of paramount importance in order to create a source of income for local populations, while alleviating the ever-increasing pressure on these precious natural resources. In this work, some

parameters affecting the success of cuttings were studied, including the nature of the substrate and the position of the cutting in the mother plant (apical and basal). The results established also showed that the herbaceous cuttings of the argan tree gave high percentages of success (50%) compared to the woody cuttings (33.3%).

For the substrate effect, we conceived vividly that the substrate 2 composed of 50% of sand and 50% of compost allowed a good recovery of cuttings by the formation of several leaves and buds as opposed to the other substrates. The low rooting rates found in our study led us to consider the use of shoots established in the herbaceous grafting technique.

The selection of clones of Argan tree with high yield, in products of quality sought after by the industrialists and their multiplication by vegetative way constitutes indeed, an important economic stake, with a considerable social impact. Advances in vegetative propagation techniques, particularly with regard to the rapid dissemination of breeding progress, are opening up new prospects in terms of the exploitation and enhancement of genetic resources. These techniques need to be generalized and popularized at the nursery level, especially for the production of improved seedlings. However, the protocols related to the various vegetative propagation techniques still need to be optimized in order to further improve the results secured at present [23].

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