academicJournals

Vol. 8(6), pp. 260-270, June 2014
DOI: 10.5897/AJPS2014.1171
Article Number: B20044B45569
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Germination tests of seeds of argan tree (*Argania spinosa* (l.) skeels) of two sources (Tindouf and Mostaganem) in the semi-arid western Algerian.

Benaouf Zohra[1]*, Miloudi Ali[2] and Belkhodja Moulay[3]

[1]Faculty of Biology, Laboratory of Ecology, Houari Boumediene University of Sciences and Technology (U.S.T.H.B), El Alia, BP 32, Bab Ezzouar, 16111 Algiers, Algeria.
[2]Laboratory of Plant Biology - Faculty of Science, University of Mascara 29000 Algeria.
[3]Laboratory of Plant physiology - Faculty of Science, University of Oran 31000 Algeria.

Received 2 March, 2014; Accepted 30 May, 2014

The Argan tree (*Argania spinosa*) is a drought-tolerant, and forest species observed in arid and semi-arid zone in Algeria, has specific ecological characteristics and many interests (forest, field, and fruit). The natural reproduction of the tree has become difficult; we have assessed the propagation method by seedlings. In our experiments, we used two seed sources collected from Tindouf and Mostaganem. Based on the results of the regeneration of the Argan tree seedlings from the laboratory, we report that soaking of seed for at least four days will certainly contribute to the success of germination. Sterilization prevents microbial contamination and improves germination. According to the results, the germination tests revealed a very high rate of germination (95%) for seeds pre-soaked in water for 96 and 120 h at 25 and 30°C. The analysis of morphological characteristics of plants under greenhouse showed that there was growth in the root system of the argan seedlings and the aerial part improved quantitatively and qualitatively. Seedlings that acclimatized are two years and above, and had a well developed and lignified air device with a large leaf, which promotes their growth. The success rate for seedlings of 12 months was very low.

Key words: Argan tree, domestication, germination, pre-soaked, acclimatization, seedlings.

INTRODUCTION

In south Algeria (Tindouf), forest based on the endemic argan tree (*Argania spinosa* L. Skeels), Propagation by seeds is the most common method used to reproduce the argan forest species. All the reforestation projects of argan in Algeria adopt this method. The most successful example of this is the Mostaganem project in coastline, which began to bear fruit after six years, and all nurseries in Tindouf which aim to rehabilitate the argan tree. The sustainability of this agroforestry system is now threatened by over-exploitation and overgrazing in Tindouf (Touaref bouaama, Targanat and Markala) by the local population and the nomads of the Polisario. This

*Corresponding author. E- mail: zbenaouf@yahoo.fr. Tel: +213776985892.

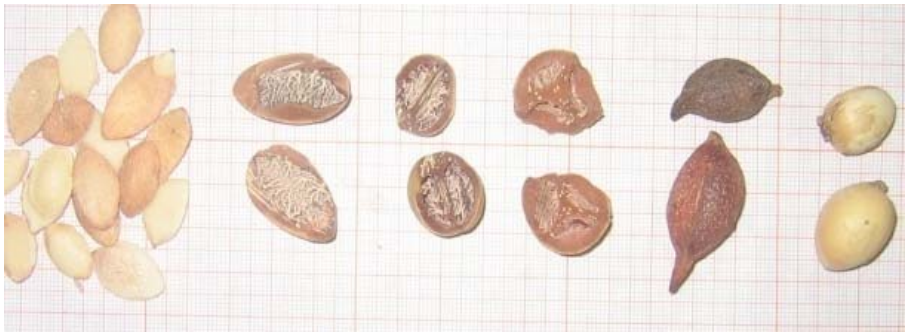

Figure 1. Components of the argan seed.

results in serious degradation of the soils and a decrease in production of oil (traditional extraction) and food betailles (camels and sheep). It is therefore a national concern for the authorities involved to protect our rare and vulnerable inherited Argan forests against anthropogenic pressure. They should allow the establishment of an executive order by the Wali of Wilaya, Tindouf bearing draft reserve natural creation (Decree No. 04/96 of 1 2/06/2004) on the protection of argan plant species in the Wilaya of Tindouf.

Perfectly suited to its environment, this endemic tree can grow on poor, shallow soils, and owing to its deep rooting system, it is considered as having a strong effect against erosion and desertification, which are the main environmental problems. The argan tree is monoecious and allogamous and exhibits high genetic diversity (Msanda, 1993). This diversity can both be preserved for ecological purposes and used through domestication. Recent initiatives have promoted the domestication of multi-purpose agroforestry species for their ability to alleviate poverty and mitigate environmental degradation (Leakey and Simons, 1998). This approach is relevant to *Argania spinosa* which could be domesticated for oil and fodder production. What is required is a package of technical procedures from the selection of the best genotypes to the production of cultivars in nurseries for successful integration into agroforestry systems. Currently, due to the failure of natural regeneration and reforestation, the only possibility of rejuvenating mature argan forest is through coppicing. Seedling production and use for plant production in nurseries could allow for the conservation of the genetic diversity. However, according to the literature, seed germination is difficult. Through selection and mass production of trees with desirable characteristics, biotechnology could improve argan tree productivity, as well as overall production (Sasson, 1993). Vegetative propagation offers the opportunity to multiply selected genotypes and to provide a significant step towards 'domestication'. But the germination is a very complex biological phenomenon which requires a good understanding and control of the factors behind. For multiplication of argan, it was found that the technique of soaking seeds in water is quite sufficient for good germination (Nouaïm and Chaussod, 1993). To overcome this constraint related to the biological nature of the seed, we performed a pre soaking in water of seeds at varying durations before planting. The purposes of the present study were: to examine the factors affecting variation in the germination of argan seeds, acclimatization of plantlets produced by germination, and to improve the success rates of nursery production and transplantation

MATERIALS AND METHODS

Propagation by seeds

The seeds used in this study came from ripe fruits collected from randomly chosen trees located in Tindouf forest (south of Algeria), and nursery of Mostaganem (coastline). The fruits were dried and their pulp was removed by hand to obtain nuts. Just before germination, they were disinfected with hydrogen peroxide for 20 min (Figure 1).

Tests for germination

The technique used was by soaking argan seeds in water at different times (72, 96 and 120 h), then the seeds were placed in the oven to heat thresholds considered (30, 28, and 25°C). Under these conditions, we want to know the ability of seeds' germination of two sources: Tindouf (natural area of argan) and Mostaganem (introduced species) (Figure 2). We conducted a test with 20 seeds for each treatment.

First germination test with a temperature of 30°C

The argan seeds were previously disinfected with bleach for 20 min; then they were pre-soaked in water and placed on cotton in Petri dishes: Treatment 1: The seeds are soaked in water for 72 h; Treatment 2: the seeds were soaked in water for 96 h; Treatment 3, the seeds were soaked in water for 120 h.

The germination tests with temperatures of 28 and 25°C

The argan seeds were disinfected prior to pure bleaching for 20

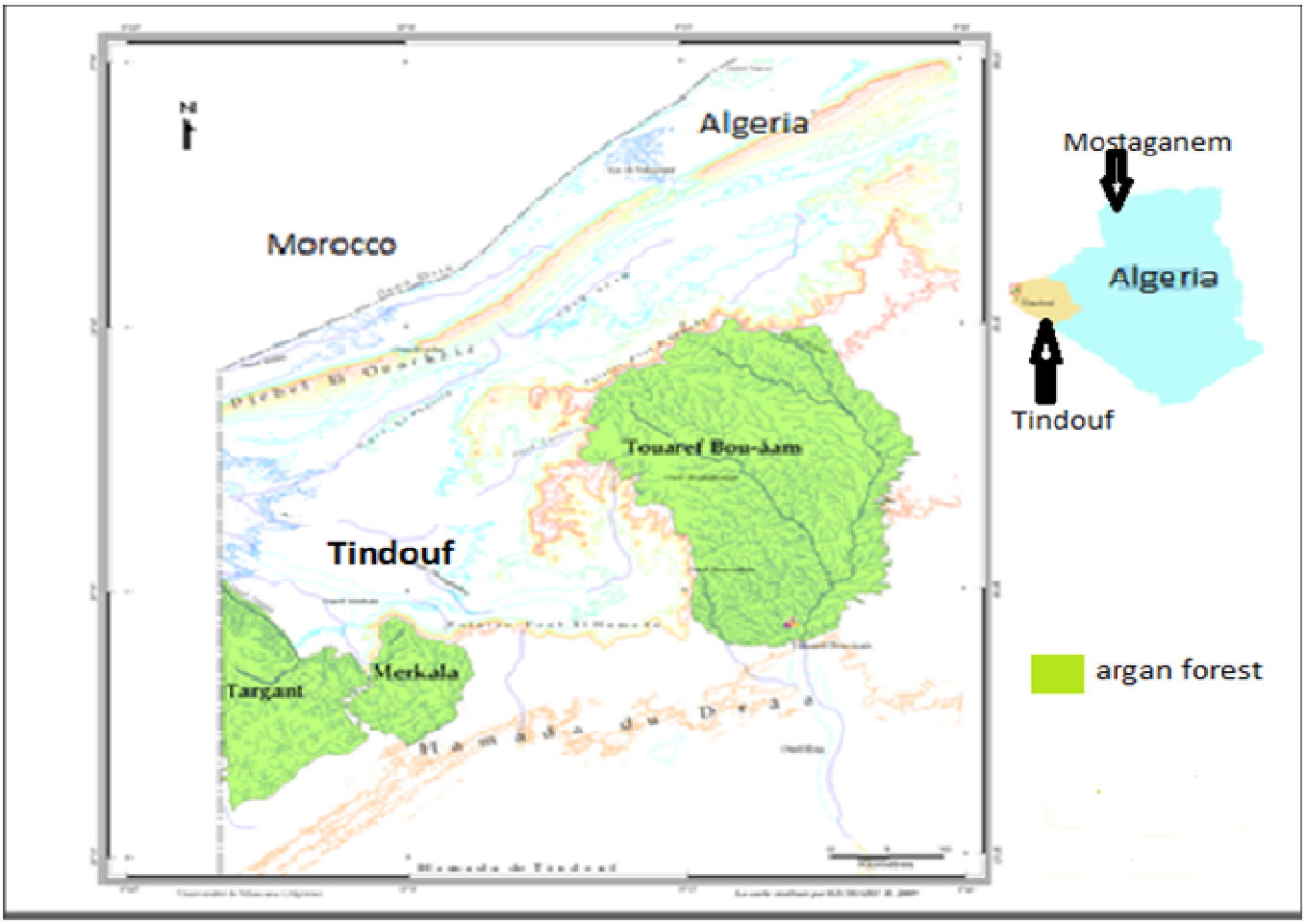

Figure 2. The whereabouts of argan in Algeria.

Figure 3. Reforestation project of argan in Mostaganem.

min, and then they pre-soaked in water treatment for 96 and 120 h only. This is because soaking for 72 h gives us very low germination than when placed on cotton in boxes kneaded (Figure 3).

Figure 4. Seedlings of argan in plastic bag.

Figure 5. Seedlings of argan in pots.

Treatment 1

The seeds were soaked in water for 96 h.

Treatment 2

The seeds were soaked in water for 120 h.

Acclimatization of seedlings

In order to know the behavior of the argan tree under the influence of abiotic conditions, we found it useful to perform planting in two different settings: Under greenhouse and open field. The choice of these two environments was done so that we can follow the growth of seedlings after planting argan and to compare the environmental conditions.

On emissions

Transplanting seedlings argan in plastic bag

In the laboratory we installed in plastic bags peat sand filled to two thirds and one-third. The experimental conditions were characterized in the laboratory by a thermal range of 18 to 24°C, exposure to sunlight and a relative humidity of ambient air of about 65 to 75%. Seedlings of the argan tree were watered every 48 h (Figure 4).

Transplanting seedlings argan in pots

Young seedlings argan obtained under the above conditions (under glass) were removed from the bags with their lumps around the roots, and then they were transplanted into pots. The substrate used consists of peat mixed with sand in equal proportion (1/2 and peat 1/2 sand) (Figure 5).

In the field

The argan tree seedlings were monitored on the ground after planting. They have the following characteristics.

Origin

These two types of argan seedlings that aged between one and two years are from the nursery of Mostaganem (littoral). We also noted that these seedlings had varying dimensions (height, diameter, branch), even those of the same age.

Planting period

Generally, planting periods are chosen depending on the rainfall. In Algeria, plantations are generally done from October to March because the soil is in good condition during that time and the humidity is high (about 90%). In our case, the argan tree seedlings were sown in 28/02/2008.

Planting method

There were 24 argan seedlings of one year and 20 seedlings of 2 years. The length of the seedlings ranged from 06-73 cm, with a number of sheets of about 150 units. A plot at the experimental farm was prepared to serve the planting site. Planting distances were 1 m between seedlings and 2 m between the lines. The first watering was done immediately after planting, then it was repeated once a month for six months after planting (the amount of irrigation water is 3 L per plant).

Statistical analysis

Both parametric (t test) and non-parametric tests (Mann-Whitney-Wilcoxon test, based on sum of ranks) were performed on experimental data for comparison of treatments (Conover, 1980).

RESULTS

Germination rate

Germination is a complex biological phenomenon that requires control and identification of factors causing it. In our case, we are interested in the argan fruit covered with a very hard shell. So we practice several treatments before sowing seeds at different temperatures to find the

Figure 6. Germination of argan seeds

optimal conditions for germination (Figure 6).

Germination tests at a temperature of 30°C

Pre soaking in water for 72 h

The seed germination started on the fourth day at a rate of 10% for those in Mostaganem against those germinated in Tindouf. Raw sproutswe observed as early as 10 days at a rate of 5%. Then, the germination rate increased slowly and reached maximum rates of 95 and 80%, respectively at duration of 20 days (Figure 7).

Pre soaking in water for 96 h

The first sprouts were obtained from the second day at a rate of 10% for both seeds. Then, the germination rate increased to a maximum of 80% on the 12th day (Figure 8).

Pre- soaking in water for 120 h

Seeds pre-soaked in water for 120 h express a relatively high germination rate (15%) from the third day of sowing: 15% for Mostaganem seeds and 10% for those of Tindouf. Germination rate increased rapidly up to 75% in seeds of Mostaganem and 80% for seeds of Tindouf on the 13th day of sowing (Figure 9).

Testing the germination temperature of 28°C

Based on the fact that the best results on germination of argan were obtained from pre soaking in water for 96 h and 120 h, we found it useful to continue the remainder of the tests with the two treatments. Seed treatment consisting of pre-soaking in water for 75 h gave very poor results.

Pre soaking in water for 120 h

Seeds pre-soaked in water for 120 h express variable responses to germination (Figure 10). Indeed for the two batches of seeds, germination begins at the third day of planting at a rate of 15% for seeds of Mostaganem and 10% for those of Tindouf. Thereafter, germination evolves to a maximum of 90% for Mostaganem seeds and 95% for those of Tindouf.

Pre soaking in water for 96 h

There was a significant difference in the behavior of the two types of seeds. Indeed, the seeds of Tindouf germinated on the sixth day and those of Mostaganem, on the fourth day, at 10 and 5% rates, respectively. As a result, germination increased to 95% for seeds of Mostaganem and 90% for those of Tindouf at day 18 (Figure 11).

Test germination temperature of 25°C

Pre soaking in water for 120 h

The first sprouts were obtained from the second day at a rate of 10 to 45% for Mostaganem seeds. Seeds of Tindouf gave a rate of 5% at the 2nd day of planting. Germination evolves rapidly to a maximum of 95% for Mostaganem seeds and 90% for Tindouf seeds on the 18th day (Figure 12).

Pre soaking in water for 96 h

There was an early germination of the second day of planting at a rate of 5% for both types of seed. As a result, the levels increase slowly and reached a maximum of 95% on the 20th day of sowing (Figure 13).

Acclimatization step

Morphological characteristics of the argan tree seedlings

Plants produced from seed had a tap root system with a fast and powerful development under these conditions: 15 days after sowing is enough for the taproot to appear through the hole at the bottom of the bags.

Main characters observations and measurements:

i) Number of leaves and thorns

Young argan seedlings had a strong capacity for growth and development. Therefore, the leaf system was significant in the first month, with an average of 30 leaves per seedling, and about 70 leaves per seedling after 9 months (Figure 14).

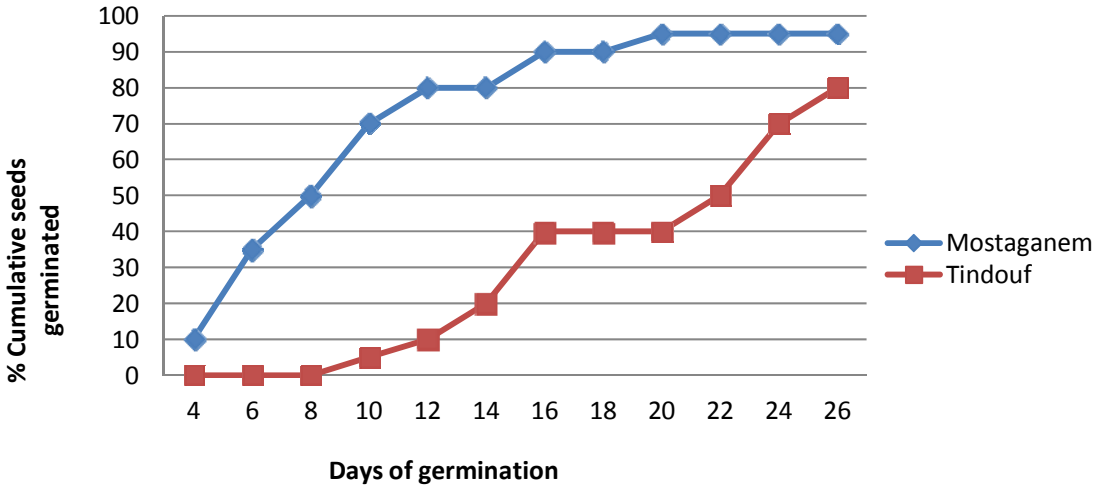

Figure 7. Daily argan seed germination rate pre-soaked for 72 h and subjected to a temperature of 30°C.

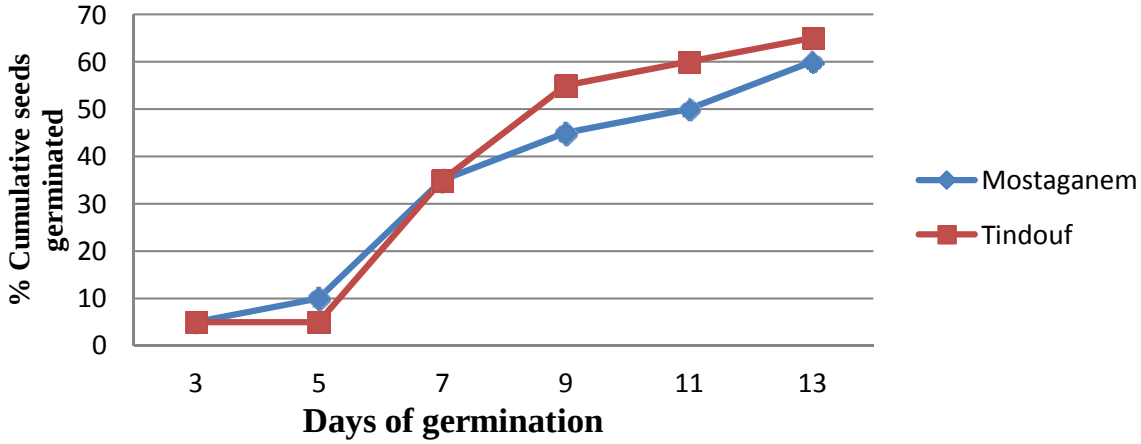

Figure 8. Daily argan seed germination rate, pre-soaked for 96 h and subjected to a temperature of 30°C.

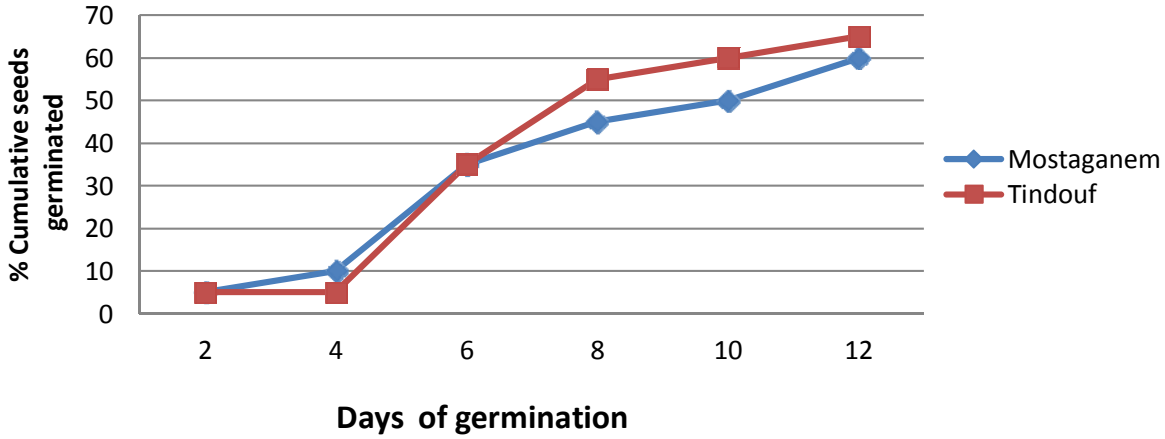

Figure 9. Daily argan seed germination rate, pre-soaked for 120 hours and subjected to a temperature of 30°C.

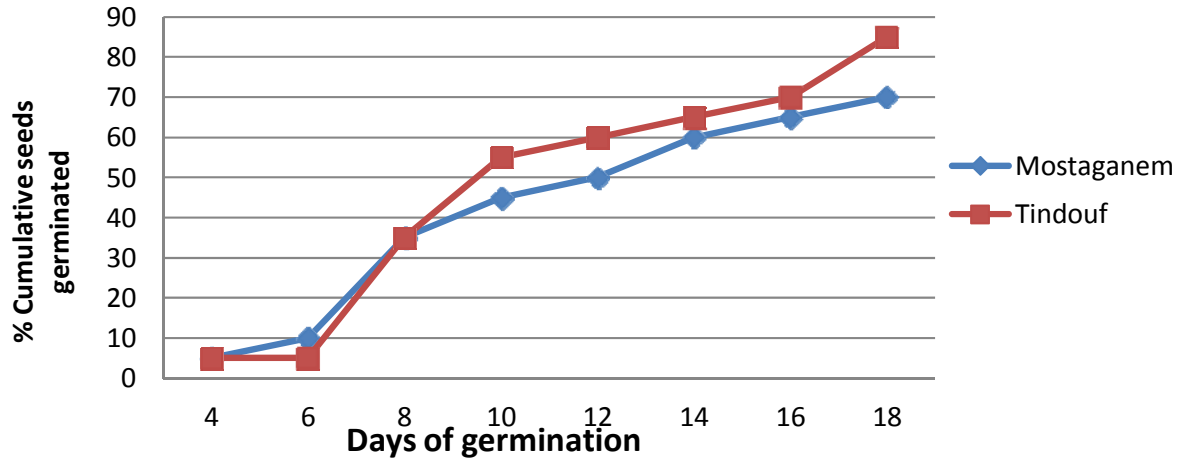

Figure 10. Daily argan seed germination rate, pre-soaked for 120 h and subjected to a temperature of 28°C.

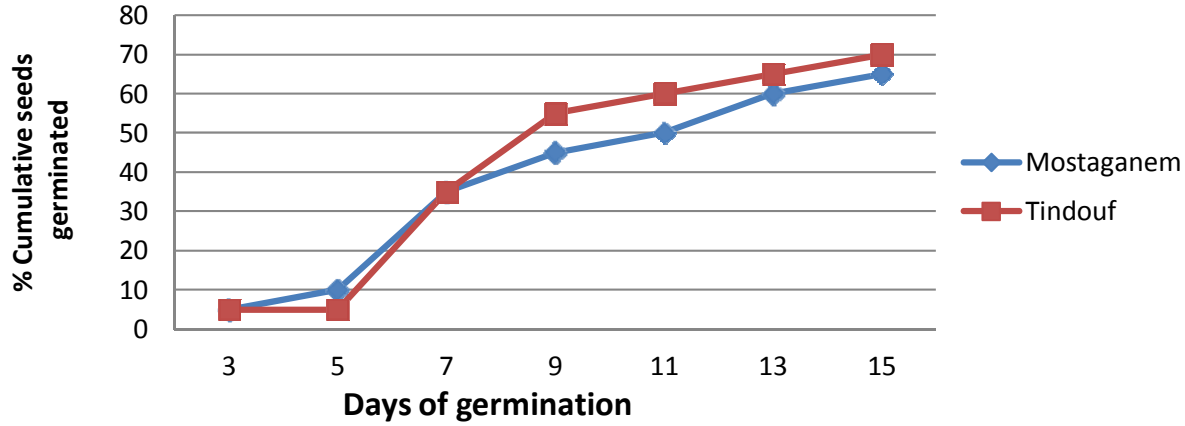

Figure 11. Daily argan seed germination rate, pre-soaked for 96 h and subjected to a temperature of 28°C.

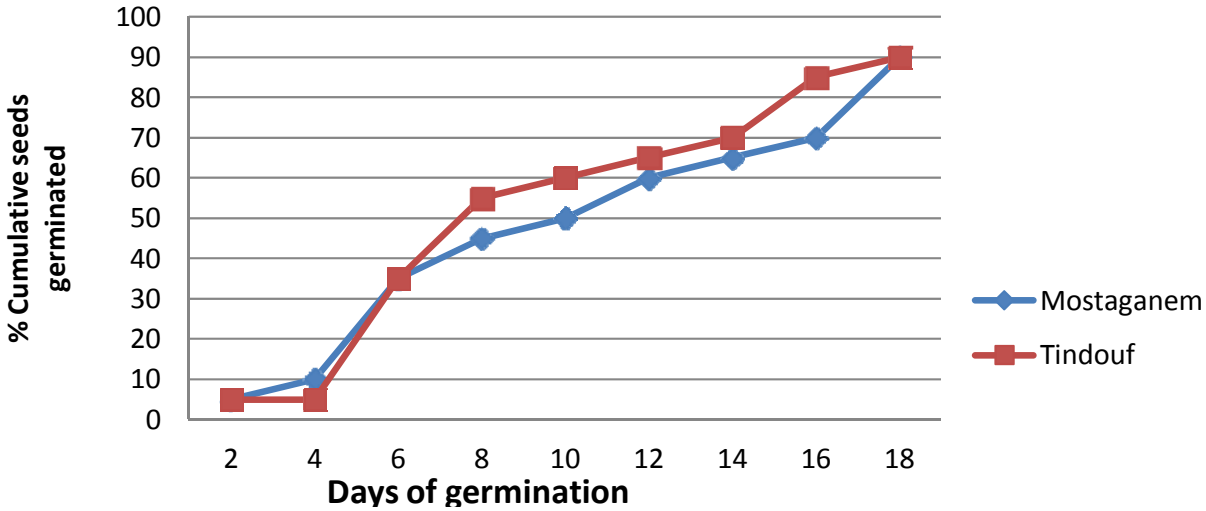

Figure 12. Daily argan seed germination rate, pre-soaked for 120 h and subjected to a temperature of 25°C.

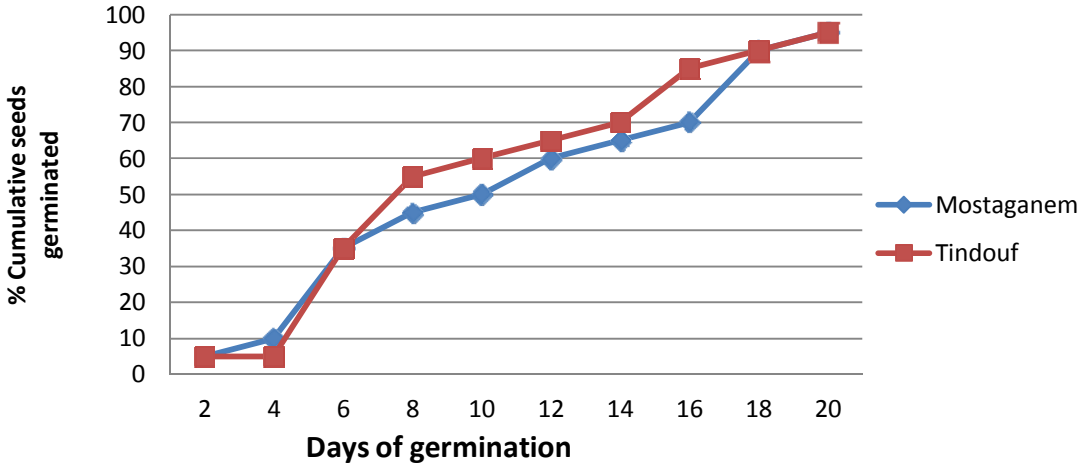

Figure 13. Daily argan seed germination rate, pre-soaked for 96 hours and subjected to a temperature of 25°C.

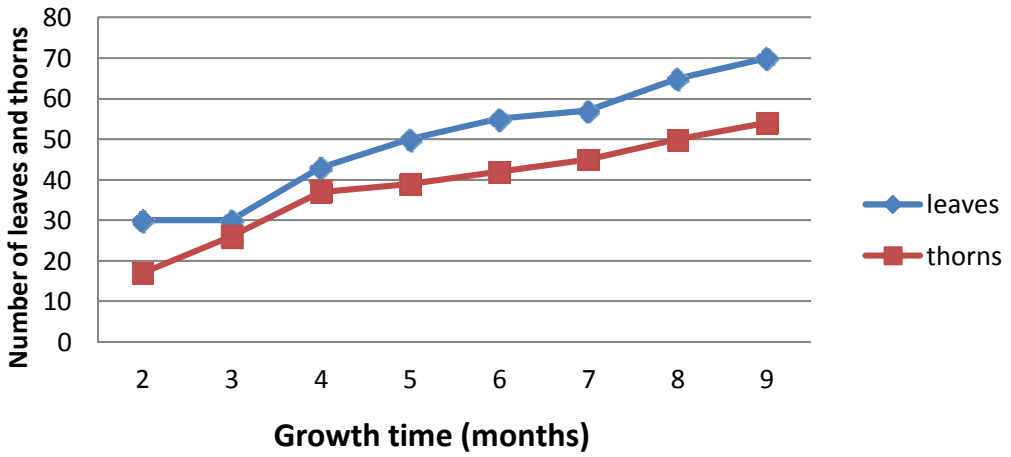

Figure 14. Monitoring the evolution of leaves and thorns of argan seedlings for 9 months.

Thorns appeared from the 20th day of the plantation, and increased with time. Thus, we noted that older seedlings of 2, 7, and 9 months haved a varying number of thorns: 17, 42, and 54, respectively.

ii) Growth in height

Height is the average height of seedlings, measured from collar to the end bud. The results show that height growth was faster in 14 days old seedlings with a height of 0.8 cm and got to 6.5 cm after a month. There is slow height growth in a month or more with two months old seedlings that have height of 18 cm and 9 months old seedlings with a height of 40 cm (Figure 15).

Results on the root system show that the length of the main root was significant; the secondary and tertiary roots were thin and brittle. After 14 days of planting, the length of the main root was 3 cm, then it underwent rapid growth to reach a length of 8 cm in a month. There was continuous growth in the root system with normal development but at a variable rate. For 4 and 5 months, the length of the primary root was respectively 25 and 29 cm. However, we observed a slowdown of root growth after 6 and 9 months; their lengths we slightly higher than the previous (32 and 35 cm). Similarly, we note that the growth in height of stem is small compared to the roots.

In the field

Height of stems

Growth in height of stem varied in the two types of

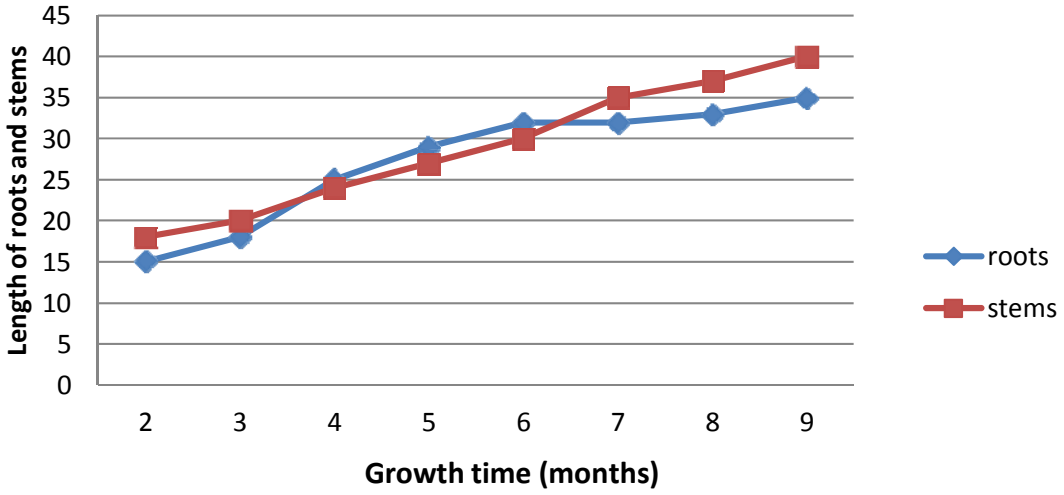

Figure 15. Monitoring the evolution of heights of stems, and roots of argan seedlings of 9 months.

seedlings. After the first week of planting, we observed that stem height has quadrupled in older seedlings of 2 years compared to one year. Then the seedlings were growing two batches of irregular height up to 74 cm (seedlings older than 2 years) and about 12 cm (seedlings older than one year) in the first six months of planting.

At the age of 12 months after planting, shoot growth slowsed down: height reachesd 18 cm respectively in one year seedlings and 32 cm for that of 2 years.

Leaf drying

Leaf desiccation is when the foliage or leaves of few seedlings dry up and become detached from the main stem. After a week when transplantation was done, the rate of drying of the leaves is very low for the two types of seedlings.

Number of thorns

From the observations, we find that the number of thorns increased with the age of seedlings; older seedlings of one year hadve 10-28 thorns each against those of two years, with 40-260 thorns each. So after a month of planting, these have the same values like previous thorns.

Branch of the stems

Young argan seedlings have an axis of stem that is rarely branched. As argan tree is vigorous, it acquires ramifications of the first order. At 2 years, the seedlings have remarkable branching of approximately 2 to 15 branches per seedling up to the 3rd month of the planting.

Rate of recovery after transplantation

The number of successful transplanted seedlings after one year, compared to results of those originally planted is seen in Table 1.

The aim of this study was to highlight the biological conditions related to promoting good regeneration of the argan tree. Our experimental tests on regeneration of seedlings argan of two years have encountered a number of climate and technical field problems, which impact negatively the development of seedlings. Then, the highest success rate was found in 24 months old seedlings (80%) compared to seedlings of 12 months (50%). This high success rate (80%) shows that the age of argan tree seedlings is significant for a good recovery.

DISCUSSION

Many authors have reported the difficulty involved the germination of the seeds of the argan tree and they recommended either scarification or acid treatment. Our experiment showed that in seeds pre-soaked in water for 120 h at 30 and 28°C, raw sprouts were obtained from the third day at a rate of 15%, as against 25 and 20°C, in which germination starts on day 3, but at a rate of 10%. Regarding the rate of germination, it was significant in seeds pre-soaked in water for 96 and 120 h in the thermal range of 30 and 25°C. Under the same conditions, in seeds pre-treated before sowing, there was optimal rate of 85% obtained at 30°C.

Regarding the origin of the seeds, the results show

Table 1. Success rate of transplanted argan seedlings.

Age (month)	Seedlings transplanted	Live seedlings	Success rate (%)
12	24	12	50
24	20	16	80

some difference in the rate and the beginning of the germination. The seeds begin to germinate in Tindouf 2 to four days late compared to those of Mostaganem. The delay in germination may be related to the different morphology of the seeds, compared to those of Mostaganem. We hold that the seeds of Tindouf have a relatively hard and thin shell.

Regarding the relationship between the germination time, temperature, and nature of the pretreated seeds source, the results indicate the existence of a correlation between the duration used for pre-soaking seeds in water and germination temperature. Moreover, the duration used in pre-soaking argan seed is very short when the optimum temperature for germination was high (30°C). The variation in germination parameters of Argan seeds we measured is reported by many authors working in the field. For example, Come (1975) reported that seed will sprout if the embryo has the possibility of imbibing; Mazliak (1982) concluded that temperature remains a limiting factor that affects directly germination by acting on the speed chemical reactions. In addition, Nouaim and Chaussod (1993) argue that the argan tree can regenerate from seeds, but a lot of failures were observed.

These authors report that a simple dipping of argan seeds in water for three or four days encourage a high percentage of germination. In our case, soaking seeds in water for 96 and 120 hbefore sowing has positive effect on early and high rates of germinated seeds (80, 85 and 95%) from 25 to 30°C. Compared to some work done in Algeria on the possibilities of multiplication of argan seeds in a thermal range of 25 to 30°C, we observe that the germination varies with sources of seeds. For example, for seeds from Tindouf, germination rate reached 50% (Slimani, 1996). This rate increaseds to 70% (Kechairi and Lakhdari, 2002) and 80% (Miloudi, 2006) for seed harvested from Oggaz Station, Mascara. For seeds from Mostaganem, germination rate reached 90% (Baoui, 2001) and 55, 70 and 80% in the thermal range of 25 to 30°C (Miloudi, 2006).

Furthermore, Renard (1975) notes that among the seeds used for the test (same experimental conditions), some do not germinate due to the hardness of the integument and others due to the endogenous inhibitors or dormancy. Under these conditions, it is essential to achieve optimal conditions for germination, confirming the general rule on the germination of halophytes (Grouzis et al., 1976), and Glycophytes (Francoit et al., 1986; Belkhodja and Soltani, 1992).

Conclusion

Failure of argan tree regeneration is often attributed to multiplication difficulties. However, our experimental results have shown that this tree can be propagated by seeds. Due to its allogamous reproduction (Msanda, 1993), different multiplication methods could be used, according to the objective. Reforestation by seedlings is the best method because it maintains the great genetic variability of the species and confers ecological resistance. It reduces production costs in forest nurseries. The aim of our work was not to define ideal methods for propagation, but to check the feasibility of this technique. Our results represent regene-ration of argan tree by seeds. This technique will be used for future production of argan trees in agroforestry systems. This points out the imbalance of traditional agroforestry system, which is now clearly threatened. The only way to avoid irreversible damage to the environment and to promote sustainable management of the argan forest is to improve agricultural income. It seems now possible to optimize argan tree based agroforestry systems, through the production of high quality exotic oil, for which there is already a large demand, exceeding present supplying capacities.

'Planting trees that yield a good profit' is the best way to fight against desertification. Leakey and Simons (1998) showed that increasing the quality, number and diversity of domesticated trees could provide a wide array of non-timber forest products (NTFPs). NTFPs could enhance the capacity of agroforestry to fulfill its ultimate potential as a way to alleviate poverty and to mitigate deforestation and land depletion. Domestication of trees for agroforestry to produce NTFPs can therefore benefit both the farmer and the environment. Vegetative propagation, enabling argan tree domestication, is a real chance for development (Sasson, 1993). We showed that argan tree domestication is technically feasible. However, dissemination of this knowledge is necessary for a true sustainable development.

Conflict of Interest(s)

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

We are thankful to the R. S.B.G. laboratory, the professors in USTHB University and Mascara University, Faculty of Biology

REFERENCES

Baoui B (2001). Test multiplication and generative transplantation argan plants (*Argania spinosa* L. Skeels) in the plateau of Mostaganem. State Engineer in Agronomy memory. Univ of Mostaganem. p. 80.

Belkhodja M, Soltani N (1992). Replies bean Vicia faba L to salinity: a study of the germination of some lines to growth. determined.Bull. Soc. Bot. FR. pp. 357-368.

Come D (1975). Some problems of terminology on seeds and their germination (Chaussâte nine Germination desemences, bordas, Paris, Bruxelles, Montréal. 1975, pp. 11-26.

Conover WJ (1980) Practical Non-parametric Statistics (2nd edition). John Wiley & sons, New York, 493p.

Francoit L, Maas E, Ovonan T (1986). Effect of salinity on grain yield, vegetative growth and germination of semi dwarf and durum wheat in an arid environment. Agro. J. pp. 1053-1058.

Grouzis M, Berger A, Heim G (1976). Polymorphism and germination in three annual species of the genus Salicornia ; OEcol. Plant. pp. 41-52.

Kechairi R, Lakhdari A (2002). Contribution to the study of the argan (Argania spinosa L. Skeels) and multiplication by seed testing laboratory in memory of engineering status in Bio. Univ of Mascara. p.120.

Leakey RRB, Simons AJ (1998). The domestication and commercialization of indigenous trees in agroforestry for the alleviation of poverty. Agroforestry Systems 38:165-176.

Mazliak P (1982). Physiologie II Plant Growth and Development. Collection methods Hermaun Paris. p. 465.

Miloudi A (2006). The physiological and biochemical responses of the argan (*Argania spinosa* L. Skeels) natural abiotic factors. PhD thesis, University of Oran ES SENIA. pp. 1-2, 30-90.

Msanda F (1993). Ecologie et cartographie des groupements végétaux d'Anzi (Anti-Atlas Occidental, Maroc) et contribution à l'étude de la diversité génétique de l'arganier (Arganai spinosa (L.) Skeels). Thèse Univ. Joseph Fourrier, Grenoble (France) 116 p.

Nouaim R, Chaussod G (1993). Argan (*Argania spinosa* (L.) Skeels). The flamboyant newsletter network members tropical trees n° 27, Septembre1993. pp. 50-64.

Renard H (1975). Germination techniques, practical criteria and significance. Chemical, biochemical criteria, physiological and molecular. Laboratory of the University of Avigon, France. pp. 24-35.

Sasson A (1993). Biotechnologies in Developing Countries: ²Present and Future. UNESCO Publishing, Paris. 764p.

Slimani H (1996). Contribution to the study of the argan (*Argania spinosa* L. Skeels) the germination of the Inland halophyte *Hordeum jubatum*. Can. J. Bot. 1420-1425.