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Eastern Morocco *Argania spinosa* propagation and growth: A follow-up study

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Abstract

The Argan tree (*Argania spinosa* L) is an endemic species, located mainly in the southwest of Morocco, but also exists as a relic in the northeast of the country. It is a species of great biological, ecological and socio-economical importance. As part of the domestication of the Argan tree at the national and regional scale, a study was carried out which aims to optimize the mode of sexual multiplication of eastern Morocco Argan tree, by studying the effect of the nuts weight on the rate of imbibition, the rate of germination, the number of seedlings resulting from each nuts classes of weight, and the growth of the seedlings. The experimental treatment is mainly formed by three classes of nuts weight. The first class named W-1 is formed by nuts with a weight less than 2g. The second-class W-2 is characterized by nuts of a weight ranging from 2 to 3.5g. The last class is W3 and has a weight of over than 3.5g. The germination was conducted in a phytotronic room at a controlled temperature (26 ± 2°C). While, the follow-up of the seedlings' growth was carried out in a glass greenhouse, and the rate and kinetics of imbibition were performed in a separate experiment. The results indicated that the nuts were water permeable and the rate of imbibition was found to be proportionally related to weight and varies between 18.18 % W1 and 32.21% W3 after 48 hours. On the other hand, for the germination rate, the nuts of class W1 showed the highest germination rate (84.4%) followed by those of class W2 (72%) and finally class W3 (68.8%). However, Argan nuts could contain more than one kernel and produce more than one seedling per nut and this allowed to have a high number of produced plants and increased the germination rate in particular for the classes W2 and W3, (99.2% and 97.2%). The results also showed that the growth and the development of seedlings were proportionally related to the weight of the kernels.

Keywords: *Argania spinosa*, Eastern Morocco, Nuts weight, Germination, Seedling growth.
Abbreviations: W-1 to 3_weight class, S-W_Seedlings weight class, GI_Growth index, S-P_Seedlings production, S-L_Seedlings length, L-N_leaves number, G-R_germination rate.

Introduction

The Argan tree or *Argania spinosa* is an endemic species of Morocco belonging to the Sapotaceae family (Peltier, 1982). This family requires a warm climate and is mostly present in tropical regions, except the Argan tree which is the only representative of this family in northern Africa. This tree contributes to the preservation of the ecosystems by promoting the existence of a floristic and faunistic biodiversity (Chakhchar et al., 2020). In addition, it plays a great biological, ecological and socio-economical importance (Mechqoq et al., 2021), and it's frequently used in traditional medicine (Kamal et al., 2021).
The distribution of the Argan tree in Morocco seems to be strongly influenced by the climate (Emberger, 1925). It was revealed the existence of the Argan tree in different geographical locations in Morocco, especially concentrated in the southwest of the country and also found in the north as a relic (*Beni-znassen, Oued-Cherrat, Plaine Bouaareg*) and this is principally due to the bioclimatically favorable conditions for to the development of *Argania spinosa* (Moukrim et al., 2018). Furthermore, the relics located in eastern Morocco are mainly found in the mountain Beni-znassen including *jbel Tikermine, Jbel Aklim, Al Guendoul, Feddane, Alhajra, Alhofra, Seffah* and near *Douar Chouihya* (Tazi et al., 2003a). This species covers an approximate area of 7.2 Km^2, with a density of 252 trees/Km^2 (Faouzi et al., 2015). On the other hand, the Argan tree is represented in the region of Nador (*Kariat Arekmane*) by 2 trees which testify to the former existence of this population in this region (Tazi et al., 2003a).
Currently, this Moroccan endemic species is in continuous regression because of its strong exploitation in silviculture as well as overgrazing. Seedling production remains a reliable process with high potential for the regeneration of plants (Tazi et al., 2003b). Sexual propagation is considered among the means to increase the diversity of the genetic heritage (Nerd et al., 1998) allowing a better adaptation to the climatic changes that Morocco is facing. The optimization of the sexual multiplication of the Argan tree remains the most

favorable choice to reach the objectives of regeneration of this species by the production of vigorous plants of good quality and with a good recovery in the natural environment. Several factors have shown a pronounced effect on the Argan tree germination rate, indeed the optimal temperature for the germination of the Argan tree nuts is between 25 and 28 °C (Berka and Harfouch, 2001; Bouzoubaâ and El Mousadik, 2003; Elmandouri et al., 2020), and preferably it should be carried out in a photoperiod of 16 hours under Light / 8 hours under dark (Alouani and Bani-Aameur, 2003). On the other hand, the germination rate may vary depending on the origin of the mother plants (Zunzunegui et al., 2013; Ferradous et al., 2017) the date of harvest of the fruits (Hamani et al., 2018), and finally the storage duration (Berka et al., 2018).

Parallelly, some pretreatments improve the nuts germination rate, such as imbibition and gibberellic acid treatments (Ikinci, 2014), mechanical scarification, and the pretreatment with the spores of some fungal species (Sellal et al., 2020). While, other operations such as chemical pretreatment by acetic acid and hydrogen peroxide seem to have an inhibitory effect on germination (Berka and Harfouch, 2001). The effectiveness of these pretreatments depends on the nuts origin (Alouani and Bani-Aameur, 2004; Zunzunegui et al., 2013). This study aims to optimize the sexual multiplication of the eastern Morocco Argan tree by studying the effect of the nuts weight on the various germination parameters as well as on the growth of the seedlings.

Results

Nuts imbibition rate

The results of the imbibition rate for the different classes are depicted in **Fig 2.**, and **Fig 3**. The water absorption by the nuts after 4 hours of imbibition was 6%, 7%, and 11% respectively for W1, W2 and W3.

The evolution of the imbibition rate as a function of time showed that the first 24 hours are characterized by a high absorption. Beyond the 24 hours, the absorption rate decreased and stabilized after the 60th hour.

On the other hand, in the germination experiment the nuts were kept in distilled water for 48 hours. **Fig 3.** shows the interpolated imbibition rate after 48 hours and it is noted that the nuts of class W3 were more permeable than the other classes, with a cumulative rate of 31.21 %, followed by the nuts of class W2 and W1 with values of 21.44 and 18.18 % respectively. The amount of absorbed water was very high for class W3 (> 1 g) while for the W1 class the amount of water absorbed was less than 0.5 g.

Germination rate

As pictured in **Fig 4.** the germination rate was inversely proportional to the weight of the nuts, and varies between 68.8% W3 and 84.4% W1. The statistical analysis (ANOVA) showed that the weight of the nuts influenced the germination rate in a highly significant way ($p<0.001$).

Rate of produced seedlings

As drafted in **Fig 5.,** taking into consideration the seedlings from the nuts containing two kernels, we note that the W3 and W2 classes compensate for their reduced germination rate by the number of produced plants per nut. The final germination rate (plants produced, **Fig 5.**) increased significantly especially in the W2 and W3 classes, (99.2% and 97.2% respectively). The analysis of variance showed a highly significant difference ($P<0.001$) between the different classes, while no difference was detected between the two classes W2 and W3.

Germination kinetics

According to the results obtained **(Fig 6.),** the W1 class tends to germinate more rapidly from 5th day , with a germination rate exceeding 25 %, followed by the W2 class and then the W3 class, which are characterized by slow germination, starting from the 10th day the W1 class exceeded 60%. While the W3 class reached a germination rate of about 25%. Finally, from the 20th day the germination rate was stable for all the studied classes

Stem length evolution according to the weight

During the first 4 weeks **(Fig 7),** the nuts of the two classes W2 and W3 showed higher growth in length than the nuts of class W1. However, during the last two weeks the results showed that the seedlings from the class W3 nuts experienced a significant change in the length of the stem. The results were confirmed by statistical analysis ($p<0.05$), which showed that the growth in length of the stem is proportional to the weight of nuts. Similarly, the seedlings from double kernel nuts **(Fig 8.)** showed a growth that was proportional to the weight of nuts. A slight difference was observed between seedlings from 2 and 1 kernel nuts for each weight class, but this difference was not statistically significant ($P>0.05$).

Evolution of the number of leaves according to the weight

Similarly, for the number of leaves **(Fig 9.)**, the results showed that seedlings from W3 class nuts had a significant change in the number of leaves compared to the other weights. The results are confirmed by statistical analysis ($p<0.05$). For seedlings from double kernel nuts of classes W1 and W2 **(Fig 10.**), they presented a low number of leaves compared to seedlings of the same weight classes but from single kernel nuts, which was confirmed by statistical analysis ($p<0.05$). On the other hand, seedlings of class W3 from double kernel nuts showed a similar number of leaves to seedlings from single kernel nuts and no significant difference was detected ($p>0.05$).

Growth index

The results of the growth index are tabulated in **Table 2**. The seedlings from W3 nuts are characterized by a very high growth rate of 1.06 cm/week, which largely exceeds the other two weight classes W2 and W1. On the other hand, the number of leaves produced is proportional to the weight of the nuts. The W3 nuts class produced the highest number 15.16 leaves/week. Concerning the seedlings from the double kernel nuts, they were characterized by slower growth in comparison with the seedlings from the single kernel nuts, in the same way for these seedlings, the growth was proportional to the weight of the kernels, of which the W3 class gave the best results.

Statistical correlation

The results of the statistical correlation study depicted in **Table 3.** showed that there is a significant statistical

Table 1. Weight class distribution of the used nuts.

Classes	Weight (g)
Classe W1	W < 2
Classe W2	2 < W < 3.5
Classe W3	W > 3.5

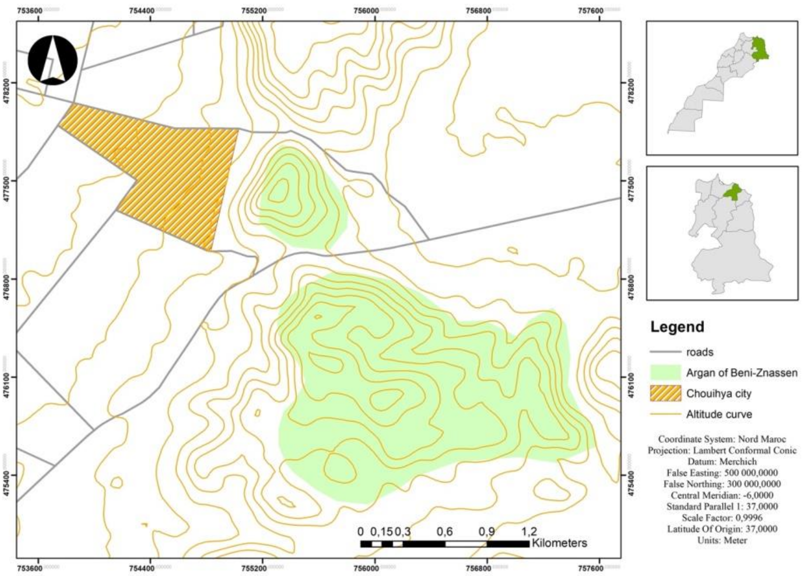

Fig 1. Geographical location of the collected fruits.

Table 2. Growth index (in stem length and number of leaves).

	Seeds class	*Growth index (cm/week)*	*Growth index (leaves/week)*
Single kernel seedling	*S-W3*	1.06	15.16
	S-W2	0.16	3.64
	S-W1	0.083	1.65
Double kernel seedling	*S-W3*	0.778	12.66
	S-W2	0.03	1.16
	S-W1	0.06	1.5

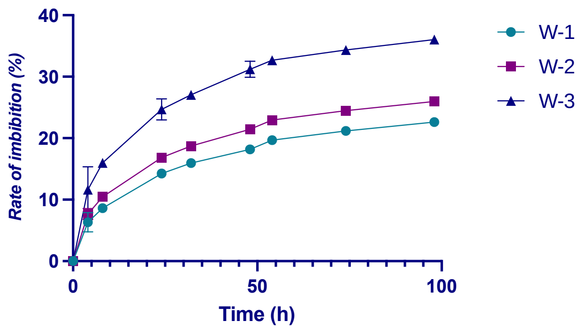

Fig 2. Permeability of nuts classes as a function of time.

Table 3. Correlation matrix between the different measured parameters.

	Weight	G-R	S-P	S-L	L-N	GI-Leaves
G-R	-0.943**					
S-P	0.817**	-0.945**				
S-L	0.850**	-0.656	0.422			
L-N	0.864**	-0.684*	0.447	0.992**		
GI-Leaves	0.926**	-0.766*	0.555	0.969**	0.981**	
GI-Lenght	0.904**	-0.730*	0.510	0.973**	0.986**	0.998**

***. Correlation is significant at the 0.01 level.*. Correlation is significant at the 0.05 level.*

GI: Growth index, **S-P:** Seedlings production, **S-L:** Seedlings length, **L-N:** leaves number, **G-R:** germination rate.

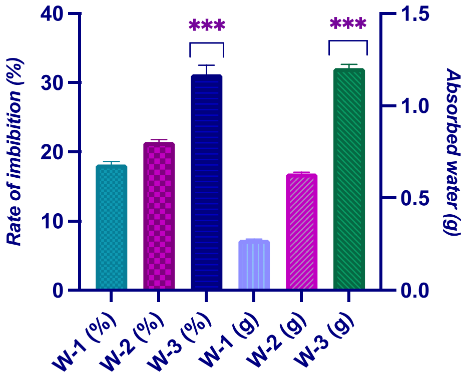

Fig 3. imbibition rate (%) and absorbed water(g) after 48 hours according to the nuts weight.

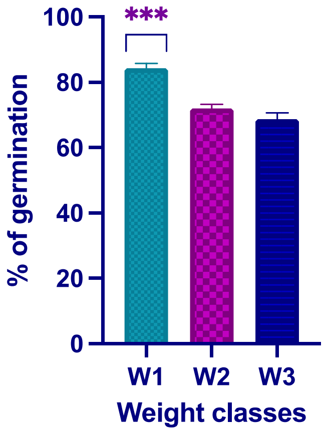

Fig 4. Germination rate according to the weight.

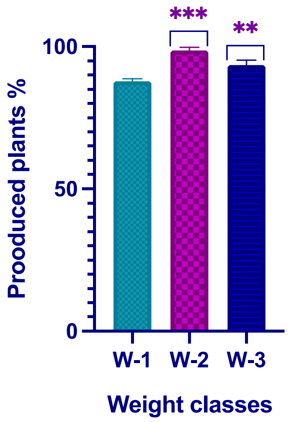

Fig 5. Rate of seedlings produced by weight class.

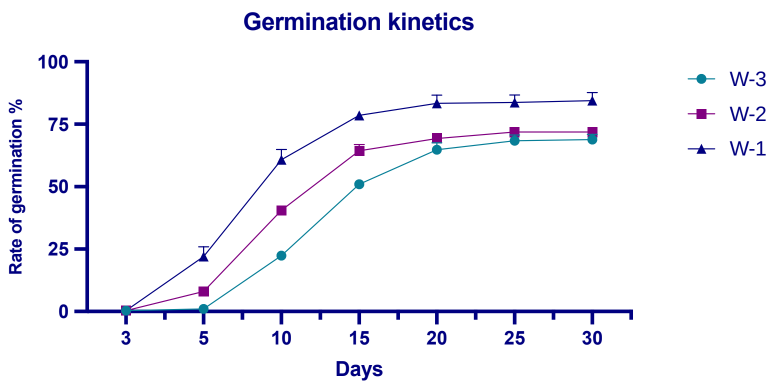

Fig 6. Germination evolution according to time.

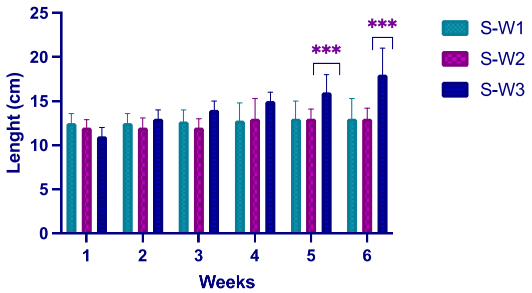

Fig 7. Length evolution of the seedlings according to weight (single kernel nuts).

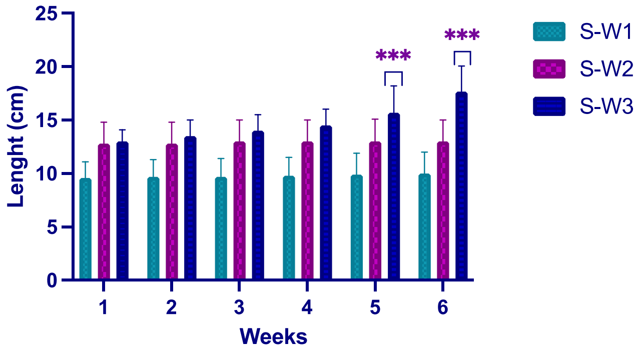

Fig 8. Length evolution of the seedlings according to weight (double kernel nuts).

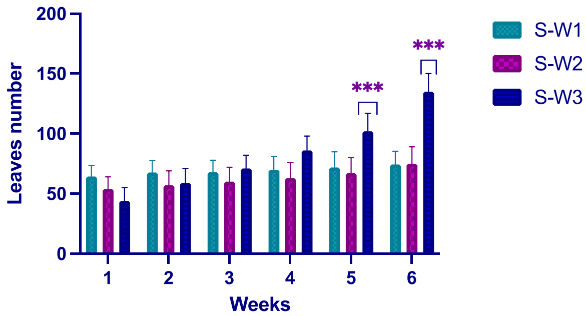

Fig 9. Evolution of the number of the seedlings leaves from single kernel nuts.

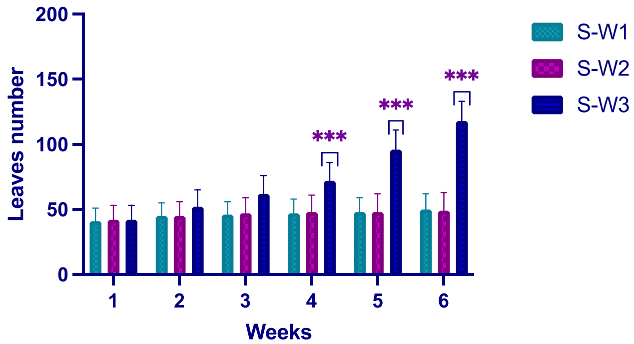

Fig 10. Evolution of the number of the seedlings leaves from double kernel nuts.

correlation between the different parameters measured. The weight of the nuts is correlated negatively with the germination rate ($p<0.01$) and positively with the length of the seedlings as well as the number of leaves and the growth index ($p<0.01$).

Discussion

The results obtained indicated that the Argan nuts weight seems to have an important effect on the germination rate. In fact, the nuts with a low weight presented the highest germination rate compared to those with a high weight. These results are different from those obtained by Reda Tazi (2003) who has indicated that the percentage of germination of the Argan nuts increases with weight. The same finding was drawn by Malcolm et al. (2003) on peach tree nuts, Upadhaya et al. (2007) on *Prunus jenkinsii*, Khan and Shankar (2001) on *Quercus semiserrata* and finally Valdés et al. (2014) on *Jatropha curcas L.* However, Nouaim et al. (2002) have reported that the germination rate in Argan tree increases with the weight of the nuts, and indicated that the nuts with a high weight will not necessarily have a high germination rate, but this high weight of the nuts may be due to the thickness of the shell while the kernel inside is very small.

On the other hand**,** Thangjam and Sahoo (2016) have stated that seeds weight of *Parkia timoriana* does not affect germination rate, which states that the relationship between germination rate and seeds weight depends on other factors including species and age of the mother plant (Mao et al., 2014), as well as the level of phytohormones and environmental conditions experienced by the plant during fruits formation have effects on seeds characteristics (Li and Li, 2016).

In our study, the number of kernels per nut improved the germination rate (production of plants per class), in fact, it reached 99.2% for class W2 instead of 72.0% and 97.2% for class W3 instead of 68.8%.

Concerning the germination kinetics, the nuts with reduced weight tend to germinate more quickly than the other classes. According to Nouaim et al. (2002), the high germination rate of small nuts is due to their thin shells.

Concerning the imbibition rate, the high weight nuts were imbibed faster than those with low weight, and after 48h of imbibition the weight classes W1, W2 and W3 recorded an imbibition percentage of 18.18; 21.44 and 31.21%, this calls into question the ideal imbibition rate for each weight class, and that the imbibition rate of 18.18% is perhaps the ideal rate to obtain a high rate and speed of germination. Beyond this value, the excess of imbibition can promote the rotting of the nuts, or also the bursting of the kernel which will generate a loss and a decrease in the germination rate.

On the other hand, our study has showed that the nuts of the Argan tree are permeable to water, and their imbibition do not require chemical or mechanical scarification, which was contradictory to the results of Bani-Aameur and Alouani, (1999) who have mentioned that the Argan nuts are characterized by an impermeability caused by the shell of the nuts, and requires mechanical scarification.

On the other hand, the growth and development of seedlings of Argan vary according to the nuts weight. Indeed, the growth of seedlings is proportional to the nuts weight and the seedlings from high weight nuts showed growth and development more important than the seedlings from low weight nuts. These results are in agreement with those obtained by Malcolm et al. (2003) who have indicated that the growth and development of peach rootstocks are proportional to the weight of the nuts, similar results were found on maize by Abd El Rahman and Bourdu (1986), and *Blutaparon portulacoides* by Cordazzo and Seeliger (2003). On the other hand, the weight of the seeds significantly affects the number of leaves, this was reported by Kolb and Steiner (1990) on the American red oak *Quercus rubra* and Valdés et al. (2014) on *Jatropha curcas.* These authors have showed that the aerial biomass of the plants is positively related to the weight of the seeds. This is probably due to the high energy stock in high-weight seeds, which allows for greater growth than seedlings from low-weight seeds (Flint and Palmblad 1978). In contrast, Chen and Maun (1999) have found that seed size and weight of *Cirsium pitcheri* did not affect seedling growth. It was noted that seedlings from single kernel nuts showed greater stem and leaf growth than those from double kernel nuts.

Material and Methods

Plant material and sampling method

The fruits used in this experiment were harvested from the Argan trees located in the Mountain of the western Beni-Znassen (*Chouihia*) which covers an area of approximately 700 ha, (Figure 1). Regarding the choice of mother trees seeds provider, a production-based sampling method was adopted for the selection of the trees. The 10 most productive trees were selected from 100 trees, and from each tree, 10 kg of fruits were taken, and then all of them were mixed.

Methods and experimental conditions

Nuts preparation: After drying the fruits in a dark place and at room temperature for 10 days. The fruits were manually decorticated, then the nuts were weighted and classified according to their weight (Table I):

Germination process: Afterwards, the nuts were disinfected with 10% bleach for 10 minutes followed by imbibition for 48 hours. This imbibition makes the hard shell of Argan nuts permeable (Berka and Harfouch, 2001), and improves the rate (Harris, 1996) and kinetics of germination (Saleem et al., 2014). The next step consists in recovering the nuts and distributing them in plastic bags containing peat for germination in a phytotronic chamber with a temperature of 26 °C ± 2 with a photoperiod of 16 hours under light / 8 hours under dark. The experimental design used for germination is the DCA (Completely Randomized Design) with 4 replicates.

Imbibition rate: Parallelly, a separate experiment was launched using the same class of nuts in order to determine the kinetics of imbibition of the Argan nuts during 100 hours. For this purpose, 10 nuts of each weight class were weighed beforehand and placed in flasks containing distilled water and the weight was measured regularly every 4 hours.

Plant growth: To monitor the growth of Argan seedlings and to determine the effect of weight on their growth, an experiment was conducted in a greenhouse using seedlings from the first experiment 6 weeks after their transplanting. The plants in each class have been chosen to be of uniform size at the beginning of the experiment. The measurement of the length of the stem and the number of leaves were carried out each week for 6 weeks.

Measured parameters

Rate of nuts imbibition: The measurements of the imbibition rate consist in calculating the difference in weight between the nuts at $T_{0,}$ and the imbibed nuts at T_i after a certain time by the following relation:

$$I\% = \left(\frac{W_{Ti} - W_{T0}}{W_{T0}}\right) * 100$$

I%: imbibition percentage.

$W_{T0-Ti:}$ weight of the nuts at time zero and i

Germination percentage (%): The percentage of germination corresponds to the number of germinated nuts compared to the total number of nuts subject to germination.

$$\%\,G = \frac{N_{GN}}{N_{IN}} * 100$$

% G = Percentage of germination

N_{GN} = number of germinated nuts

N_{IN} = number of initial nuts

Kinetic of germination: It is mainly based on counting weekly the number of germinated nuts, and of calculating the rate of germination according to time.

The number of produced plantlets: Argan nuts could contain more than one kernel and produce more than one seedling per nut. This parameter is about calculating the number of plants produced by each class of weight, and of calculating their percentage according to the number of initial nuts.

Growth of seedlings in length (cm) and the number of leaves: consists of measuring the length of the stems as well as the counting of the leaves every week.

Growth index: Correspond to the rate of growth in length and number of leaves per week:

$$S_{GI} = \frac{SL_{Ti} - SL_{t0}}{ED}$$

S_{GI}= Stem growth index
SL = stem length at Time i and Time 0
ED = experiment duration (in weeks)

$$L_{GI} = \frac{LN_{Ti} - LN_{t0}}{ED}$$

L_{GI} = leaf number growth index
LN = leaf number at the time I and time 0
ED= experiment duration (in weeks)

Statistical analysis

The results were subjected to an analysis of the variance (ANOVA), using the software "SPSS for Windows version 23" followed by the Tukey test with Post Hoc multiple comparisons threshold of 5%. All experiments were carried out in triplicate, and data were expressed as the mean ± SD.

Conclusion

According to the results, the weight of the Argan nuts affects the rate of germination as well as the growth and development of the seedlings. The highest weight of nuts is the most important criteria which gave the highest values as well for the production of plants as for the growth of the seedlings. Also, It should be noted that W1 class nuts had the highest germination rate compared to the W2 and W3 classes. The latter compensated their reduced germination rate compared to class W1 by the number of seedlings produced. In fact the final germination rate for classes W3 and W2 is 99.2% and 97.2% respectively. On the other hand it was shown that the nuts of the Argan tree are permeable to water, and do not require scarification, in addition the imbibition rate of 18.18 % recorded by the W1 classes after 48 hours of imbibition seems to be ideal to trigger germination. On the other hand, to obtain this imbibition rate for the other classes, the W2 class must be imbibed for 32 hours and the W3 class for 24 hours. In conclusion, these results imply the consideration of nuts weight and the imbibition time during the production of Argan seedlings, in order to have vigorous plants, of good quality and with a good adaptation in the natural environment.

Conflicts of interest: The authors declare no conflicts of interest.
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