Range Mgmt. & Agroforestry 39 (1) : 72-76, 2018
ISSN 0971-2070

Effect of harvest time and seed coat on germination of *Argania spinosa* (L.) Skeels

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Received: 14th October, 2017 Accepted: 26th May, 2018

Abstract
The argan tree is very important to the areas it occupies. However, its natural regeneration is quasi-absent, and reforestation is achieved through planting. To study germination in response to improving different factors, we collected argan fruits from Southwest Algeria. We found that the harvest period had a significant effect on seed germination (P<0.001) and that late harvesting and seed storage at 4°C resulted in poor germination potential. Additionally, shelled seeds required a short latency period to germinate. Further, the mother tree had no significant effect on the germination percentage. Data suggested that an earlier harvest date is likely to be beneficial for seedling production.

Keywords: Argan, Germination, Harvest date, Longevity, Southwest Algeria

Introduction
The argan is a tree endemic to Algerian-Moroccan that belongs to the *Sapotaceae* family and is a forage, fruit and forest species. In Algeria, the characterization, enhancement, and promotion of production of this biological material have not been studied in recent years (Benaouf *et al*., 2014; Errouane *et al*., 2015; Hachem *et al*., 2016). Argan tree is a good forage source throughout the year, especially in drought periods, due to the palatability of its leaves for the goat population. Unfortunately, the current state of the argan tree is a real concern, as there was no natural regeneration due to human activity and severe drought in the region. This situation necessitates the preservation and rehabilitation of argan forests. Cutting, grafting, layering and transplanting the seedlings helps in the regeneration of this species. Among these methods the last one (seedlings) helps in maintaining high genetic variability in the population (Nouaim *et al*., 2002), because this mode of reproduction requires cross-pollination and is probably an important source of resilience for this species (Echairi *et al*. 2008). It was, therefore, necessary to study this regeneration mode where germination is the key step in the development cycle. Several studies showed the influence of environmental conditions on seed germination of different plants (Khera *et al*., 2000; Rajora *et al*., 2006; Bhuker *et al*., 2013). In this study, we aimed to know the effect of key factors that regulate germination including harvest period, seed conservation, seed coat and the effect of the mother tree.

Materials and Methods
Seed source: Ripe argan seeds were collected from Oued Elma (Touaref Bouam) which is located in the city of Tindouf in Southwest Algeria. The collection site is located at an altitude of 500 m above mean sea level and at 8°00'-3°40' latitude and 25°30'-29°40' longitude. The region had a Saharan climate with minimal annual rainfall, resulting in drought throughout the year (Fig 1), high average temperatures, and wide fluctuations in day and night temperature. The soil in the study area was sandy loam.

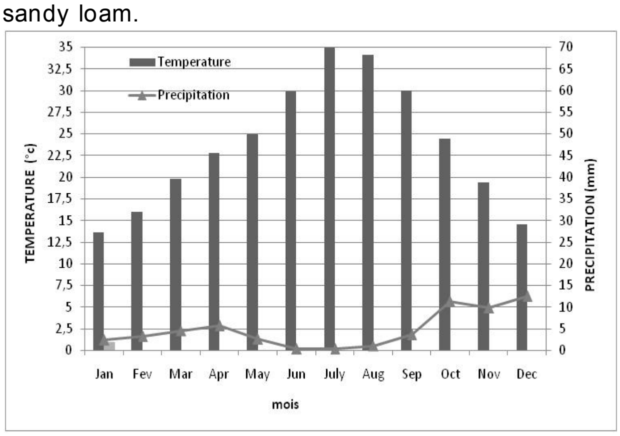

Fig 1. Climate of the study area

Seed preparation and germination tests: For each harvesting period, seeds were surface sterilized using 2% sodium hypochlorite solution for 15 minutes followed by three rinses with sterile water. We tested two replicates of 30 seeds each for germination on filter paper with a sub layer of wet cotton in 20 cm glass petri dishes. The ûlter papers were rewetted regularly with distilled water as and when required. The dishes were placed in a controlled environment chamber at 25 ± 1°C. The germination percentage was recorded daily till 45 days to study the seed vigour.

Experimental design: The seeds from mature fruits harvested in mid-June, mid-July and mid-August from 2012 to 2015 were taken for the study. To study the effect of shell on germination**,** the coats of specimens were carefully removed from the 2015 harvest and studied the germination of the kernel. The germination of 3-year-old seeds harvested in June and stored at 4°C using standard method was also tested for the effect of seed storage on viability. The harvests of six individual mother trees located at a distance of 100 m from each other were compared to determine whether the mother tree had an effect on seed germination.

***Statistical* analysis:** The data of germination percentage was analysed following analysis of variance (ANOVA) after applying arcsine transformation. The statistical analysis was done using R (version 3.2.3) software.

Results and Discussion

Effect of harvest period: Variations of germination percentages for different harvest months were recorded (Fig 2). The lowest germination percentage was recorded for seeds harvested in August, but the germination increased with the early harvest. Harvest month significantly affected the percentage of seed germination (P <0.001; Table 1). Indeed, samples from later-developing seeds recorded the lowest percentage germination. The seeds showed varying degrees of dormancy. The germination percenrage varied from 53.3 to 86.7%, depending on the month and year of harvest. The corresponding variation in the latent time was 2 to 12 days and the time to reach the germination plateau was 36 to 42 days.

Table 1. Germination (%) of argan seeds harvested over four years

Year	Months		
	June	July	August
2012	80.0	70.0	53.3
2013	73.3	63.3	53.3
2014	86.7	73.3	66.7
2015	76.7	66.7	53.3

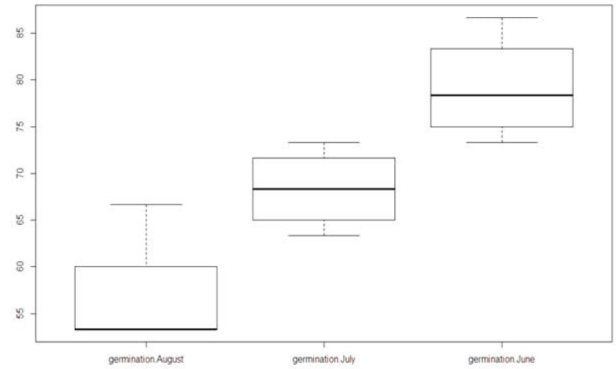

Fig 2. Boxplots for variation in germination percentage for seeds harvested at different months

Harvesting time also influenced the proportion of germinated seeds during different 10-day periods (Fig 3). It was observed that June seeds were characterized by the highest frequency of precocious germination, with frequencies of 20.83 and 35.83% after the first and second 10-day periods of germination, respectively.

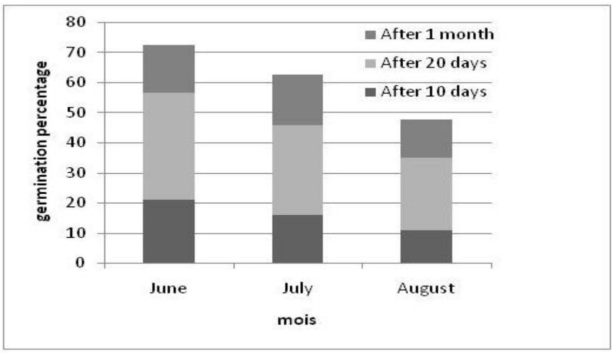

Fig 3. Germination precocity depends on the harvest period

Effect of shell: The removal of the seed coat improved seed germination parameters. Deshelled seeds germinated earlier and faster than whole seed (Fig 4). The latent period for deshelled seeds was shorter (2 to 6 days), because seed coat was water-resistant. The germination time depends on how long it takes water to penetrate the seed coat. Although removal of seed coat allowed moisture to reach the seed more quickly, it made the naked kernels susceptible to pathogenic attacks and led to decay during germination. Thus, the seed coat was essential for preventing fungal attacks.

Effect of seed storage: Initially there was no difference in germination percentage of freshly harvested and stored seed. Freshly harvested seeds had a slightly higher germination (79.7%) than the 1 to 3 year-old seeds stored at 4°C. The process of seed germination during the stor-

-age was also recorded (Fig 5). Our evaluation of germination capacity of freshly harvested seeds showed that seeds were initially viable, after 1 year of storage, this viability decreased and germination percentage declined from 76.7 to 70%. With each year of storage, germination was declined by approximately 6%. The storage of dry seeds at low temperature (4°C) showed more interesting results. Two year-old seeds harvested in June had better germination quality than fresh seeds harvested in August.

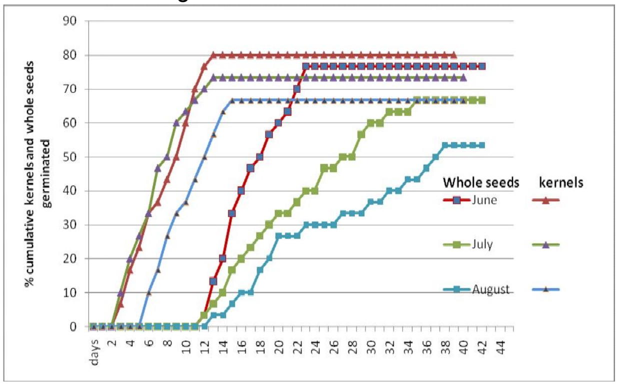

Fig 4. Germination curves for kernels (deshelled seeds) and whole seeds

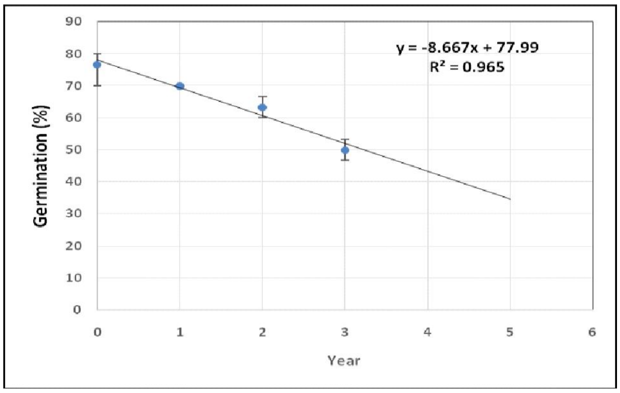

Fig 5. Effect of storage duration (4°C) on final seed germination

Effect of mother tree: Differences in germination percentage between mother-trees were not significant (Table 2), this result suggested that mother tree has no influence on seed quality in terms of germination. Propagation of argan tree is possible by both sexual and asexual methods. The nut contains one to three almond-shaped kernels, which increases the potential for sexual reproduction. Thus, argan seed germination is hindered by the hard seed coat. Additionally, it is possible that the seed dormancy was associated with the non-viability (Zahidi and Bani-Aameur, 1997; Bani-Aameur and Alouani, 1999). Argan trees spread their germination over a longer time period, this germination heterogeneity might be an important ecophysiological strategy for adaptaion to environmental heterogeneity, because dormancy provides a strategy for seeds to germinate over time and allows some seeds to avoid unfavorable environments such as drought. Argan seed coat has been implicated to be a barrier to water absorption. However, water is required to hydratate proteins and cell organelles; it acts as a medium for transport and as a substrate for hydrolytic reactions.

Table 2. One-way ANOVA of the effects of harvest month, mother tree and seed on germination

Source of variation	Df	S.S	M.S	F-Value	Pr (>F)
Harvest month	2	2026	1013.2	14.4	0.000115***
Mother tree	5	285.2	57.05	0.629	0.686
Seed conservation	3	388.7	129.6		

***(P<0.001)

During the seed-filling phase, the source-sink relationship depends on environmental conditions, including temperature and rainfall (Burstin *et al*., 2007; Lemoine *et al*., 2013). These conditions exert a strong maternal effect on the size and quality of seeds produced (Valencia-Dýaz and Montana, 2005). It was further revealed that seeds collected in different months, had different seed weights, and that ressource allocation varied among early fruits and those that matured at the end of season (El-Keblawy *et al*., 1996; Galloway, 2002). It was observed that early harvesting overcame seed dormancy and increased the germination percentage of argan seeds. Consequently, the harvest month of argan seeds had predicative value for germination. The effect of conservation was not significant, yet there was a decline in germination as storage durations increased. These results are similar to those obtained by Alouani and Bani Aameur (2014) for the argan seeds as well as for Jatropha seeds (Bhuker *et al*., 2013). Indeed, temperature is considered as the most important factor affect enzyme activities that influence seed conservation (Marques *et al*., 2014).

Argan seed dormancy is due to presence of hard seed coat. The seed coat not only restricts water and oxygen uptake, but also acts as a mechanical barrier for radicle protrusion (Debeaujon *et al*., 2000). In the present study, it was observed that removing the seed coat enhanced the germination to 80 % and reduced the latent time irrespective of harvesting period. The hard seed coat tissue contains cellulose in thick lignified cell walls that make it impermeable to water and oxygen (Sebaa and Kaid Harche, 2014). The seed coat-associated dormancy effects could be reduced by mechanical scarification (Allouani and Bani-Aameur, 2014). Another method to interfere with physiological dormancy mechanisms involves soaking the seeds for 4 days in water before germination, which facilitates water absorption and hastens germination. These effects are attributed to enzyme activation and swelling and softening of seed coat (Mudasir *et al*., 2012). Although the removal of the seed coat resulted in faster germination, the seed coat remains a means to protect against fungal attacks during germination. Fungi belonging to the genera *Fusarium* spp*., Aspergillus* spp. and *Penicillium* spp. isolated from seeds could cause decaying of seeds (Alouani and Bani-Aameur, 2014).

The seed coat has a major influence on seed germination, vigor and longevity, which depend on the integrity of the seed coat (Francisco *et al*., 2001). The seed coat is responsible for the transmission of environmental cues to the interior of the seed, and allows the embryonic plant to make metabolic adjustments for the external environment (Radchuk and Borisjuk, 2014). The marginal populations of this monotype genus are present in the Algerian Western Sahara between the Ouarkziz Mountain and the Hammada of Tindouf (Peltier, 1983). These argan trees have very similar germination characteristics, and have unique resistance capabilities because they occupy the most marginal and driest station/habitats.

Conclusion

There are numerous factors that can influence germination, including time of harvest. Our results indicated that early harvesting of seeds maximises germination and reduces the occurrence of other problems in developing seeds. Germination ability can be further enhanced by removing the seed coat. Additionally, seed storage at 4°C helps in maintaining its viability up to 3 years.

Acknowledgement

The authors are thankful to all those who directly or indirectly helped in conducting the present study and special thanks to forest sector of Tindouf city for providing the seeds of the argan tree species.

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