Journal of Experimental Agriculture International

41(6): 1-6, 2019; Article no.JEAI.53518
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Cloning of *Azadirachta indica* A. Juss. Using Juvenile Cuttings

Álvaro Renan Vieira Nunes[1], Eder Ferreira Arriel[1], Marcelo Soares Pimentel[1], Maria José de Holanda Leite[2*], Samara Paulo dos Santos Fernandes[1], Erik Alves Bakke[1], Mellina Nicácio da Luz[1] and Leonardo Castro Arriel[3]

[1]Federal University of Campina Grande, Patos, Paraíba, Brazil.
[2]Federal University of Alagoas, Rio Largo, Alagoas, Brazil.
[3]State University of Paraíba, Campina Grande, Paraíba, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. Authors ARVN and EFA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MSP, MJHL, SPSF, EAB, MNL and LCA managed the analyses of the study. Author MSP managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/v41i630439
Editor(s):
(1) Dr. Mohamed Fadel, Professor, Microbial Chemistry, Department Genetic Engineering and Biotechnology, Division National Research Center, Egypt.
Reviewers:
(1) Mary-Louise Mhazo, Training Mananga Regional Centre, Eswatini.
(2) Jayath P. Kirthisinghe, University of Peradeniya, Sri Lanka.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53518

Original Research Article

Received 25 October 2019
Accepted 27 December 2019
Published 04 January 2020

ABSTRACT

Azadirachta indica A. Juss. of the Meliácea family originates from India. It is considered important in Brazil due to its multiple uses. The plant is usually propagated by sexual reproduction (seeds), however, after harvest the seeds have to be sown as soon as possible because they lose germination viability very quickly. The use of juvenile propagules for *A. indica* seedlings propagation is a viable option, since there is a high demand in the semi arid regions due to wide use in urban afforestation. The objective of this research was to evaluate the effect of fertilization regimens and environments on obtaining Apical cuttings of juvenile origin and on the quality of cloned seedlings of *Azadirachta indica.* The research was carried out at the Forest Nursery of UFCG/Patos-PB, Brazil, with vegetative propagules (cuttings) obtained from three environments and two fertilization regimes: Biweekly and monthly, in addition to the control (without fertilization). The experiment was arranged in completely randomized design, factorial 3 x 3 (environments of origin of cuttings x fertilization regimes), with six replications, where each plot consisted of a

**Corresponding author: E-mail: maryholanda@gmail.com;*

cutting, totaling 54 experimental plots. Results indicated that propagation of *Azadirachta indica* through cuttings of juvenile origin is feasible, with an average rooting of 95.2%. It is recommended to use the monthly fertilization regimen, since in general it did not differ significantly from the biweekly regime. Fertilization provided better development and quality of the root system and aerial parts.

Keywords: Clonal propagation; mineral nutrition; semiarid; indian nim.

1. INTRODUCTION

Azadirachta indica of the Meliaceae family is popularly known as nim in Brazil, England, Spain and Portugal. In these last two countries it is also known as margosa. In Australia and the United States it is known as Neem and, in Africa it is know by various names such as nim, babo, yaro and marrango. In India its country of origin, it is known as Neem, nim and limba [1].

This meliáceae has been standing out all around the world, due to its multiple of uses [2,3] the extracts are used as insecticide, acararicides and nematicide. The multipurpose uses of neem has resuted in the plant receiveing special attention from researchers.

The main method used for the propagation of *Azadirachta indica* is sexual reproduction (seeds). However, after harvest the seeds have to be sown as soon as possible because they lose seed viability very quickly. In addition, the seeds have great potential for other uses, such as oil extraction in combating agricultural pests, manufacture of shampoos, hair oils, nails, veterinary use, among others [4,5,6]. This demand can further aggravate the availability of seeds for seedling production.

Since demand for seedlings is great in semiarid region due to wide use on urban afforestation, the use of juvenile propagules for seedlings production becomes an excellent alternative [7,8]. Its rapid development, ornamental beauty, great canopy formation providing a comfortable shade, plus greater resistance to natural enemies are some of the main reasons for this demand.

Internal and external factores affect rooting success of the vegetative propagules. These factors are inherent to the mother plant and the environment. Among the external factors that can influence cloning to obtain a good quality change are mineral nutrition during the development of cloned seedling.

In view of the above, cloning of the plant through propagules of juvenile origin is an alternative that must be tested, aiming at obtaining seedlings to meet the growing demand, in addition to the use of all the other advantages that the cloning technique provides.

The aim of this study was to evaluate the effect of fertilization regimens and environments of obtaining apical cuttings of juvenile origin on the quality of cloned seedlings of *Azadirachta indica*.

2. MATERIALS AND METHODS

The research was carried out at the Forest Nursery of the Universidade Federal de Campina Grande (UFCG), Patos Campus, Paraíba State. The predominant climate in the region is hot semiarid type, classified as Bsh. The average temperature is higher than 25.5ºC and average annual rainfall of 728 mm. The experiment was installed in completely randomized design, factorial 3 x 3 (environments of origin of cuttings x fertilization regimes), with six replications, where each plot consisted of a cutting, totaling 54 Plots.

Fruits were collected in adult trees of *Azadirachta indica* located on the UFCG Campus of Patos-PB. The seeds were extracted and availed according to procedures recommended by Nunes et al. [9]. Sowing was done in plastic tubes with 5 cm diameter and 15 cm in length, with approximately 280 cm^3 of volume. The tubes were filled with medium granulometry vermiculite as substrate, packed in polypropylene trays and allocated in suspended beds at 1 m high.

This environment (A1) was protected with screen that retains 50% of the light intensity and with controlled irrigation system, being automatically irrigated at one-hour intervals for one minute between 7 and 17 hours. After emergence, 54 seedlings were transplanted in PET bottles with approximately 1550 cm^3 of substrate composed of soil (50%), manure (25%) and Plantmax®(25%).

After 15 days, 18 seedlings remained in this environment (A1) and 18 seedlings were transferred to another screened environment that retains 50% of the light intensity, and irrigation was performed three times a week (A2) and 18 seedlings were transferred to an environment in full sun with irrigation performed three times a week (A3), resulting in 18 seedlings in each environment.

After reaching approximately 35 cm high, the seedlings were severed 15 cm from the apex in order to break the dormancy of adventitious buds stimulating the emergence of lateral shoots, resulting in the formation of a miniclonal hedge consisting of 18 mini-stumps, in each environment.

The apical branches resulting from the hew downs were used in this experiment to get apical cuttings about 12 cm in length. Eighteen cuttings were obtained from each environment, totaling 54 apical cuttings in the three environments. In this experiment no fertilization was performed in the first three months. In the last two months two fertilization regimens were used: biweekly (R1) and monthly (R2), in addition to absolute control, without fertilization, (R3). Six cuttings of each environment were used by fertilization regime, ending the experiment at 150 days.

They were added in each tube, with intervals according to the fertilization regimen, 1.5 grams of Vitaplan® macro and micronutrients with the following formulation: 8% total nitrogen (N), 9% phosphorus (P2O5), 9% potassium oxide (K2O), 3% calcium (Ca), 2% of sulfur (S), 1% Magnesium (Mg), 0.03% Boron (B), 0.005% Cobalt (Co), 0.2% Copper (Cu), 0.2% Iron (Fe), 0.005% Molybdenum (Mo) and 0.35% Zinc (Zn).

The tube, substrate and environment (A1) for the planting of cuttings used were the same for seed germination, because these conditions are favourable for rooting. At 150 days after planting the cuttings, height data (cm) were collected using a graduated ruler, Seedling lap diameter (mm)_using a digital caliper and the number of rooted cuttings to obtain the rooting percentage.

The aerial part of the seedlings was removed using a pruning shears and the roots removed from the substrate and washed. The aerial parts and the roots were packed in paper bags and placed in a sterilization and drying oven at 65 ± 0.5ºC for approximately three days until it reached constant mass. After drying, the roots dry mass (RDM, g) and the shoot dry mass (SDM, g), it was_determined in a semi-analytical scale with accuracy of 0.001 g. Finally, the total dry mass (TDM, g) and dry mass of roots and dry mass of shoot ratio were calculated (RDM/SDM).

The quality of the seedlings was evaluated by the Dickson Quality Index (DQI), according to Fernandes et al. [10].

For the rooting percentage variable, the Chi-Square - X^2 test was applied to the significance level of 5% and the other data submitted to ANOVA and the Scott-Knott test, at the significance level of 5%.

3. RESULTS AND DISCUSSION

The overall rooting average was (95.2%) (Fig. 1). This high percentage of rooting observed can be explained due to the use of vegetative material from the juvenile phase of the plant [11]. For the variable rooting percentage, it was found that there was no significant interaction of the source ambient factor of the cuttings and fertilization regime of the seedlings of *Azadirachta indica* cloned and there was also no significant effect between the environments cuttings and between fertilization schemes. The non-significance of the interaction and also between fertilization regimens was expected because these fertilizations were performed from the fourth month, when it is difficult the possibility that there are unrooted cuttings still alive.

The importance of fertilization independent of the cuttings source environment (Table 1) was verified for the height of the seedlings. A significant interaction of the source environments factor of cuttings and fertilization regimen for height and diameter was observed. Significant differences were not observed for the diameter of the cuttings originating from environments 2 (A2) and 3 (A3), but in absolute values to those fertilized were higher.

The overall average of the diameter independent of the factors studied was 3.42 mm, slightly higher than the 3.28 mm found by Lima [12], also at 150 days after staking.

Fertilization is an extremely important practice for the supply of nutrients in an appropriate and balanced way, for the production of seedlings, especially nitrogen (N) and potassium (K) because they are nutrients required in greater quantities for growth and plant development [13].

It is observed that there was a significant effect of the fertilization regime for RDM in A1, and the Control is overcome by 1.25 g in relation to the biweekly fertilization regime (Table 2). In other environments, there were no significant differences between the three fertilization regimens.

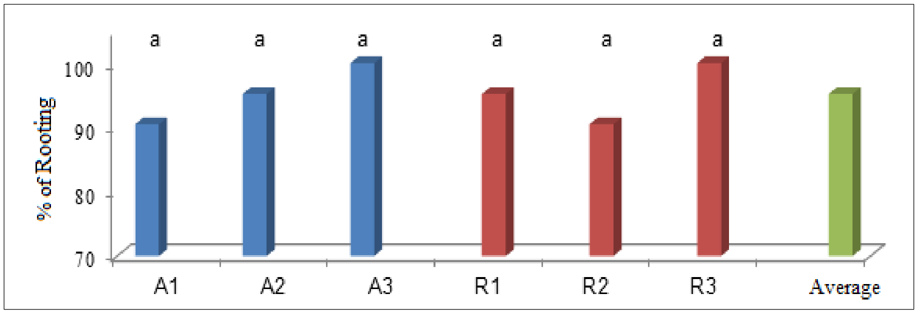

Fig. 1. Effect of the source environment (A1, A2 and A3) and fertilization regime (R1, R2 and R3) on the percentage of rooting of *Azadirachta indica* cuttings 150 days after planting

** Averages followed by the same letter do not differ from each other by the Chi-Square test (X^2), at the significance level of 5% (p > 0.05)*

Table 1. Effect of the source environment (A1, A2 and A3) and the fertilization regime (R1, R2 and R3) of *Azadirachta indica* cuttings in the height and diameter of the lap of the cloned seedlings at 150 days after planting

Fertilization schemes	Height (cm)			Diameter (mm)		
	A1	A2	A3	A1	A2	A3
R1 - Biweekly	16,3 a	13,3 a	08,6 a	4,00 a	3,51 a	3,35 a
R2 - Monthly	12,3 a	10,2 a	09,6 a	3,86 a	3,31 a	3,40 a
R3 - Control	08,2 b	05,9 b	05,2 b	3,26 b	3,13 a	2,92 a

**Averages followed by the same letter in the column do not differ from each other by the Scott-Knott test, at the significance level of 5% (P > 0.05)*

Table 2. Effect of the source environment (A1, A2 and A3) and the fertilization regime (R1, R2 and R3) of *Azadirachta indica* cuttings in the dry mass of the root (RDM), shoot dry mass (SDM) and total dry mass (TDM) of cloned seedlings at 150 days after planting

Fertilization schemes	RDM (g)			SDM (g)			TDM (g)		
	A1	A2	A3	A1	A2	A3	A1	A2	A3
R1 - Biweekly	4,72 a	4,80 a	3,84 a	4,00 a	5,20 a	3,96 a	08,72 a	10,00 a	07,80 a
R2 - Monthly	4,04 b	4,06 a	4,52 a	3,86 a	4,28 a	4,32 a	07,90 b	08,34 b	08,84 a
R3 - Control	3,47 b	4,05 a	3,75 a	3,26 b	3,35 b	3,39 b	06,73 b	07,40 b	07,14 a

** Averages followed by the same letter in the column do not differ from each other by the Scott-Knott test, at the significance level of 5% (P > 0.05)*

Table 3. Effect of the source environment (A1, A2 and A3) and fertilization regime (R1, R2 and R3) of *Azadirachta indica* cuttings in the relationship between root dry mass and shoot dry mass (RDM/SDM) and Dickson Quality Index (DQI) of cloned seedlings at 150 days after planting

Fertilization schemes	RDM/SDM			DQI		
	A1	A2	A3	A1	A2	A3
R1 - Biweekly	0,81 a	0,92 b	0,96 a	1,99 a	2,05 a	2,17 a
R2 - Monthly	0,52 b	0,94 b	1,04 a	1,99 a	2,02 a	2,34 a
R3 - Control	0,85 a	1,20 a	1,10 a	2,04 a	2,13 a	2,66 a

** Averages followed by the same letter in the column do not differ from each other by the Scott-Knott test, at the significance level of 5% (P > 0.05)*

The cuttings from the three environments, shoot dry mass (SDM) differed statistically (Table 2). The control was significantly different from the biweekly and monthly fertilizer regime which were singnificantly similar). Rosse [14], working with clones of *E. dunnii* generated by propagules of juvenile origin, and fertilized with nitrogen, found mean dry mass values of 1.58 g per mini-cutting. When compared to the present study these values are low, because the lowest value obtained in fertilization treatments for the MSPA was approximately 3.86 g. This may have occurred due to the potential of the species easy adaptation to the environment and also due to the fertilization regimen which not just nitrogen.

Table 3 shows the results of the relationship between root dry mass and shoot dry mass (RDM/SDM) and Dickson Quality Index (DQI).

Quality seedlings must present an RDM/SDM ratio equal to or greater than 0.5 [15]. The values found in the present study are well higher than this value and also superior than those found by Fernandes et al. [10] who found an RDM/SDM relationship of less than 0.50, in a research with this same species, but with an evaluation much earlier, at 120 days. According to the authors, one of the possible causes of these low values observed is the short time for the development of roots, notably because it is a system of development of adventitious roots that is slower when compared to the seminal system. This corroborates the present research that evaluated RDM/SDM for this cloning system for a longer period of seedling development (150 days).

Ferraz & Engel [16] report that a low RDM/SDM ratio can influence the ability to establish individuals in the field, and may cause the tipping of the same, since they have a limited root system and shoot Protuberans. Silva [17] considers that the result of this relationship should be complemented by the analysis of biomass data. It is verified, therefore, that in general, regardless of the environment that the propagules were collected, the seedlings that received fertilization had values of RDM, SDM and TDM higher than the control (Table 2).

Regarding the Dickson Quality Index (DQI) no significant variation was detected between fertilization regimens in *Azadirachta indica* seedlings originated from the three juvenile propagule collection environments (Table 3).

Caldeira et al. [18] classify 0.20 as minimum value for DQI for evaluation of seedling quality. In this study the high values of the DQI in all situations analyzed (Table 3) confirm that the time of evaluation of seedlings was sufficient for the formation of cloned seedlings of good quality, as well as the size of the propagule used, corroborating the arguments of Fernandes et al. [10], regarding the time and size of the propagule used for the production of cloned seedlings of *Azadirachta indica.*

It is important to note that in cloned seedlings of *Azadirachta indica* the two factors studied (environment of origin of cutting and fertilization regimens) although they did not influence the percentage of rooting, they had significant effects on the development and quality of rooting, that is, in the quality of the seedling, which are extremely important for the survival and development of the same in the field.

4. CONCLUSIONS

The propagation of *Azadirachta indica* A. Juss through use of juvenile cuttings, is feasible, resulting in a high percentage of rooting, with an average of 95.2%.

Monthly fertilizer regime did not differ significantly from the biweekly regime thus cost may be reduced by using monthly fertilization. The efficiency of fertilization in the quality of seedlings provides a better development and quality of the root system and shoots, notably verified by the height and biomass production of cloned seedlings that received fertilization with macro and micronutrients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vilela JAR. Effect of dermal neem oil (*Azadirachta indica*) and subcutaneous moxidectin use on the prevention of Dermatobia hominis infestation (Linnaeus JR., 1781) (Diptera; Cuterebridae) in cattle. Dissertation (Master in Veterinary Medicine) - Rural Federal University of Rio de Janeiro (UFRRJ), Seropédica, RJ. 2008;53.
2. Fernandes IJ, Correia TR, Ribeiro FA, Cid YP, Tavares PV, Scott FB. Effectiveness of neem (*Azadirachta indica*) in the control of Demodex canis (Leydig, 1859) in dogs. Brazilian Journal of Veterinary Medicine. 2010;32(Suppl.1):59-63.
3. Igarashi M, Carvalho DMC, Bucci FC, Miranda, Rodrigues ZM, Almeida MCF, Iona MNM. Effect of neem (*Azadirachta indica*) on the control of gastrointestinal nematodes in sheep supplemented with dry pasture. Semina: Agricultural Sciences, Londrina. 2013;34(1):301-310.

4. Wengrat APGS, Uemura-Lima DH, Barilli DR, Gazola D, Fredrich JE, Ringenberg R, Pietrowski V. Efficiency of azadiractin-based product in the control of lacebug nymph in cassava crop. Agroecology Notebooks, Pinhais, PR. 2014;9(1):5.
5. Silva MCA. Rosa LS, Vieira TA. Efficiency of neem (*Azadirachta indica* A. Juss) as a natural barrier to attack by *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) on mahogany (*Swietenia macrophylla* King). Acta Amazonica. 2013;43(1):19-24.
6. Ferreira ECB, Gonçalves SG, Bicho CL. Efficiency of neem products applied to stored peanut pods on *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Green Magazine (Mossoró - RN).2014;9(2):07-09.
7. Lacerda MA, Soares FS, Costa JPM, Medeiros RS, Medeiros EN, Carvalho JA, Silva ZL. Floristic survey of urban afforestation in the main public roads of the municipality of Boa Ventura, PB. Brazilian Journal of Environmental Management - RBGA, Pombal - PB – Brazil. 2013;7(4):12–16.
8. Moraes LA, Barbosa RRM. The Urban afforestation of the city of Timon, MA: inventory, diversity and quali-quantitative diagnosis. Journal of The Brazilian Society Of Urban Forestation, Piracicaba – SP. 2014;9(4):80-98.
9. Nunes ÁRV, Arriel EF, Ramos GG, Almeida EP, Silva RPS. Production and survival of *Azadirachta indica* miniceps A. juss. in different environments and fertilization regimes. Re. C.E.F, Heron, SP. 2017;30(1):53-62.
10. Fernandes SPS, Arriel EF, Almeida EP, Araujo AN, Arriel DAA, Justino STP. Cutting height for establishment of neem clonal mini-garden (*Azadirachta indica* A. Juss). Patos-PB: Scientific Farming in the Semiarid. 2017;13(1):67-71.
11. Souza-Junior L, Quoirin M, Wendling I. *Grevillea robusta* A. Cunn. from juvenile propagules. Forest Science. 2008;18(4): 455-460.
12. Lima FS. Production of minicuttings in neem minicaps (*Azadirachta indica* A. Juss) submitted to different manage-ment systems. Monograph (Undergraduate in Forest Engineering) - Federal University of Campina Grande, Paraíba. 2016; 37.
13. Ferreira KS, Rufini JCM, Fagundes MCP, Moreira SG, Ferreira EVO, Barbosa MAP. Nutrient growth and accumulation in acerola seedlings due to the application of different doses of nitrogen and potassium. Colloquium Agrariae. 2019;15(2):37-50.
14. Rosse LN. Estimates of genetic and phenotypic parameters of shoot re-growth and rooting capacity in Eucalyptus spp. Dissertation (Master in Genetics and Plant Breeding) - Federal University of Lavras (UFLA), Lavras, MG.1995;77.
15. Daniel O, Vitorino ACT, Alovisi AA, Mazzochin L, Tokura AM, Pinheiro ERP, Souza EF. Application of phosphorus to Acacia mangium willd seedlings. Tree Magazine, Viçosa, MG.1997;21(2):163-168.
16. Ferraz AV, Engel VL. Effect of tube size on the quality of jatoba (*Hymenaea courbaril* L. var. Stilbocarpa (Hayne) Lee et Lang.) Seedlings, yellow ipe (*Tabebuia chrysotricha* (Mart. Ex DC.) Sandl.) and guarucaia (*Parapiptadenia rigida* (Benth.) Brenan). Tree Magazine, Viçosa, MG. 2011;35(3):413-423.
17. Silva MG. Use of vermiculite by-products in the production of saplings of caatinga tree species. Monograph (Undergraduate in Forest Engineering) - Federal University of Campina Grande, Paraíba. 2015;36f.
18. Caldeira MVW, Gomes DR, Gonçalves EO, Delarmelin WM, Sperandio HV, Trazzi PA. Biosolid in the substrate composition for the production of Tectona grandis seedlings. Forest Magazine, Curitiba, PR. 2012;42(1):77-84.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53518