Original Research Article

CLONING OF *Azadirachta indica* A. Juss. BY THE CUTTING TECHNIQUE OF JUVENILE ORIGIN

ABSTRACT

Azadirachta indica A. Juss. of the Meliácea family originates from India, it has been standing out in Brazil due to its multiplicity of 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 power very quickly. An alternative to work around this problem is the use of propagules of juvenile origin for the production of seedlings of *Azadirachta indica*, because the demand is great in the semiarid region mainly due to its wide use in urban afforestation. The objective of this research was to evaluate the effect of fertilization regimens and environments of obtaining apicais cuttings of juvenile origin on the quality of cloned seedlings of *Azadirachta indica*. The research was developed at the Forest Nursery of UFCG/Patos-PB, Brazil, with vegetative propagules (cuttings) obtained from three environments and applied fertilization with macro and micronutrients in two fertilization regimes: Biweekly and monthly, in addition to the control (without fertilization). The experiment was installed in completely randomized design, arranged in factorial scheme 3 x 3 (environments of origin of cuttings x fertilization regimes), with six replications, where each plot consisted of a cutting, totaling 54 experimental plots. The propagation of *Azadirachta indica* through cuttings of juvenile origin is feasible, having presented an average percentage of 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.

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 by various names such as nim, babo, yaro and marrango and in its country of origin, India is known as Neem, nim and limba (VILELA, 2008).

This meliáceae has been standing out all around the world, due to its multiplicity of use (FERNANDES et al., 2010; IGARASHI et al, 2013) and the importance of its extractives that are

Comment [MM1]: Can have title as "Cloning of Azadirachta indica A. Juss using juvenile cuttings" instead of By the cutting technique.....

Comment [MM2]: Full stop

Comment [MM3]: Rewrite as: It is considered important in Brazil due to its multiple uses.

Comment [MM4]: They loose germination viability ....instead of germination power

Comment [MM5]: Begin sentence as "The use of juvenile propagules for A. indica seedlings propagation is a viable option

Comment [MM6]: Rewrite as "since there is high demand in the semi arid regions due to wide use in urban afforestation"

Comment [MM7]: Replace of with on

Comment [MM8]: Apical

Comment [MM9]: Insert the word and on the quality of cloned seedlings.....

Comment [MM10]: Was carried out instead of developed

Comment [MM11]: Not necessary in abstract since it should be fully explained in methods

Comment [MM12]: Arranged instead of installed

Comment [MM13]: Delete arranged

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Comment [MM15]: Delete scheme

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Comment [MM17]: Begin as "Results indicated that the propagation of A. indica....

Comment [MM18]: Write as "with an average rooting of of 95.2%

Comment [MM19]: It is known by various names such as nim.....

Comment [MM20]: Put full stop, Delete and. Begin new sentence "In India its ocountry of origin it is known as neem, nim and limba

Comment [MM21]: Due to its multiple uses, the extracts are used as insecticides, acaricides and nematicides

related to use as insecticide, toxines to kill ticks and nematicide. And due to this multipurpose this plant has received special attention from researchers.

The main method used for the propagation of *Azadirachta indica* is by sexual reproduction (seeds). However, after harvest the seeds have to be sown as soon as possible because they lose germination power 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 (WENGRAT et al., 2014; SILVA et al., 2013; FERREIRA et al., 2014). This demand can further aggravate the availability of seeds for seedling production.

The use of propagules of juvenile origin for the production of seedlings of *Azadirachta indica* would be an excellent alternative, because the demand for these seedlings is great in the semiarid region mainly due to its wide use in urban afforestation (LACERDA et al., 2013; MORAES & BARBOSA, 2014). 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.

There are factors that directly affect the success of rooting vegetative propagules, which are internal and external factors, that is, inherent to the mother plant and the environment. Among the external factors that can influence cloning to obtain a good quality change we have 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*.

MATERIAL AND METHODS

The research was developed 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 temperature is higher than 25.5ºC and average annual rainfall of 728 mm.

Fruits were collected in adult trees of *Azadirachta indica* located on the UFCG Campus of Patos-PB. The seeds were extracted and benefited according to procedures recommended by NUNES et al. (2017). Sowing was performed on plastic tubes with 5 cm diameter in extremity and 15 cm in length, with approximately 280 cm^3 of volume, filled with medium granulometry vermiculite as substrate, packed in polypropylene trays and allocated in suspended beds at 1 m high.

Comment [MM22]: See comment 20

Comment [MM23]: No sentence starts with the word and. Begin sentence as "The multipurpose uses of neem has resulted in the plant receiveing special attention from researchers

Comment [MM24]: Delete by

Comment [MM25]: Replace germination power with seed viability

Comment [MM26]: Since demangd 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.

Comment [MM27]: See comment 25, rewrite sentence

Comment [MM28]: Rewriteas: Internal and external factores affect rooting success of the vegetativepropagules. These factors are inherent to the mother plant and the environment

Comment [MM29]: See comment 27

Comment [MM30]: Delete and replace with "are"

Comment [MM31]: Why upper case C and T

Comment [MM32]: Specify the environments i.e 3 environments named

Comment [MM33]: Use carried out at the Forest .....instead of developed

Comment [MM34]: Include the location in terms of latitude

Comment [MM35]: Is this average temperature or mazimum temeperature

Comment [MM36]: See comment 58

Comment [MM37]: Get a clearer term. Benefited might be misleading

Comment [MM38]: Sowing was done in than performed

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This environment (A1) is 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, the transplant of 54 seedlings was performed for PET bottles with volume of 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 for the installation of this experiment with the manufacture of 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 were the same used for seed germination, due to these conditions favor rooting. At 150 days after planting the cuttings, height data (cm) were collected with the aid of a graduated ruler, Seedling lap diameter (mm) with the aid of a digital caliper and the number of rooted cuttings to obtain the rooting percentage.

Then the aerial part of the seedlings was removed with the aid of pruning shears and the roots removed from the substrate and washed. The air part and the roots were packed in paper bags and placed in a sterilization and drying oven at 65 ± 0.5 ºC for approximately three days until it reached constant mass. After drying, it was determined the roots dry mass (RDM, g) and the shoot dry mass (SDM, g) 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

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Comment [MM43]: After emergence 54 seedlings were transplanted in PET bottles with approximately 1550 cm3

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Comment [MM46]: To get apical cuttings of 12 cm. Delete with manufacture of

Comment [MM47]: What was added in each tube, specify

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Comment [MM50]: Using a graduated than with the aid

Comment [MM51]: Using a digital caliper. See comment 48 above

Comment [MM52]: Delete and begin with "The aerial parts

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quality of the seedlings was evaluated by the Dickson Quality Index (DQI), according to Fernandes et al. (2017).

The experiment was installed in completely randomized design, arranged in factorial scheme 3 x 3 (environments of origin of cuttings x fertilization regimes), with six replications, where each plot consisted of a cutting, totaling 54 Plots.

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%.

RESULTS AND DISCUSSION

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 overall rooting average was (95.2%) (Figure 1). This high percentage of rooting observed can be explained due to the use of vegetative material from the juvenile phase of the plant (SOUSA JUNIOR et al., 2008).

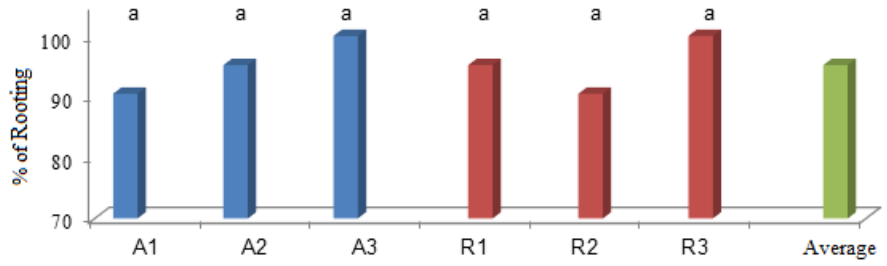

Figure 1. Effect of the source environment (A1, A2 and A3) and fertilization regime (R1, R2 and R3) on the percentage of rooting of *Azadirachta indica* cuttings 150 days after planting.
* Averages followed by the same letter do not differ from each other by the Chi-Square test (X^2), at the significance level of 5% ($p > 0.05$).

A significant interaction of the source environments factor of cuttings and fertilization regimen for height and diameter was observed. The importance of fertilization independent of the cuttings source environment (Table 1) was verified for the height of the seedlings. 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.

Comment [MM58]: Other references are in upper case

Comment [MM59]: Move to beginning of methods. See comment 35

Comment [MM60]: Should mention results first therefore this should be first sentence on results and discussion

Comment [MM61]: Move to beginning of paragraph befor explaining non significance nature

Comment [MM62]: Results always come first before discussion

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 (2016), also at 150 days after staking.

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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 (FERREIRA et al., 2019).

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	**Height (cm)**			**Diameter (mm)**		
schemes	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).

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.

In the cuttings originated from the three environments, the setomas of the shoot dry mass (SDM) of the biweekly fertilization regime (R1) did not differ statistically from the monthly fertilization regime (R2), but differed from the control (Table1). Rosse (1995), 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 was not just nitrogen.

Comment [MM64]: The cuttings from the three environments dry shoot mass differed statistically (Table 2). The control was significantly different from the biweekly and monthly fertilizer regime which were singnificantly similar

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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	**RDM (g)**	**SDM (g)**	**TDM (g)**

schemes	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 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 (DANIEL et al.,1997). The values found in the present study are well higher than this value and also superior than those found by Fernandes et al. (2017) 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 (2011) 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 (2015) 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 none of the three juvenile propagule collection environments (Table 3).

Caldeira et al. (2012) classify 0.20 as minimum value for DQI for evaluation of seedling quality. In this study it is possible to affirm that the high values of the DQI in all situations analyzed (Table 3) also reinforce 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. (2017), regarding the time and size of the propagule used for the production of cloned seedlings of *Azadirachta indica.*

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.

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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).

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.

CONCLUSIONS

The propagation of *Azadirachta indica* A. Juss through cutting of juvenile origin, it is feasible, with a high percentage of rooting, with an average of 95.2%.

It is indicated the use of the monthly fertilization regime, since in general it did not differ significantly from the biweekly regime, reducing the cost. 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.

Comment [MM75]: Through use of juvenile cuttings is feasible resulting in a high percentage of rooting

Comment [MM76]: Delete it

Comment [MM77]: Sentence not clear.

Comment [MM78]: Monthly fertilizer regime did not differ significantly from biweekly regime thus cost may be reduced by using monthly fertilization

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