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RESEARCH ARTICLE

VEGETATIVE PROPAGATION BY AERIAL LAYERING OF PTEROCARPUSERINACEUS: IN THE SUDANIAN ZONE

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ABSTRACT

Researches on the development of vegetative propagation techniques have been carried out on Pterocarpuserinaceus trees in Burkina Faso. The objective of the study is to develop low cost production techniques of plants by air layering. It is specifically to highlight the diameter of the branch, the positions of the layers on the branch and the frequencies of watering which optimize the rooting rate of the layers. Two trials were carried out in rainy seasons in Saria, Burkina Faso, on a 10-year-old *P. erinaceus* plantation, where the mean height was 10.7 ± 2.5 m. A device with two treatments has been set up. This involves making air layer at 3 different positions of the branch (apical, median and maximum) and for each position, to consider three (3) classes of diameter. The first trial, set up in 2013, consists of 126 air layers with 3 positions on the branches and 3 classes of diameter per position. The secondtrial, set up in 2014, is composed of 100 air layers with 3 positions on the branches and 2 classes of diameter per position. Air layers were watered every 20 days and weekly during the first and second trial respectively. After two months of monitoring, the air layers were unpacked and the appeared roots were measured. The results show that the rooting rate is higher in the proximal air layers with a diameter between 2 and 3 cm (76.4%), followed by the laying of the medial position with a diameter between 1 and 2 cm (52 , 9%). The supply of a quantity of water of 40 ml every week is the frequency of watering which optimizes the rate of rooting. Air layering is an alternative way for the production of *P.erinaceus* plants.

INTRODUCTION

The Sahelo-Sudanian zone is characterized by irregular and / or insufficient rainfall and characterized by recurrent droughts such as those of 1972/73 and 1984/85 (Ganaba *et al*., 2005). These crises are manifested by soil depletion and degradation, resulting in a decline in NTFP production and crop yields (FAO, 2003). In addition, the Sahelian zone is the northern limit of the distribution zone of several plant species including *Vitellariaparadoxa*, *Parkiabiglobosa*, *Prosopisafricana*, *Bombaxcostatum*, *Combretumnigricans* and *Pterocarpuserinaceus* (Arbonnier, 2004). Belong to the family Fabaceae, *P. erinaceus*Poir. is part of the useful plant species which are currently overexploited and threatened in West Africa. This species is highly sought by craftsmen in Niger, Mali and Burkina Faso for manufacturing various arts objects and musical instruments (Ouédraogo *et al*., 2006, Rabiou *et al*., 2015). *P. erinaceus* Poir. is also widely used in traditional medicine (Ouédraogo *et al*., 2011). At the end of the dry season, this species is covered by new leaves and flowers as herbaceous fodder are becoming scares. The trees are then pruned by the herders to feed their herds. The tree can no longer produce fruit, thus its regeneration is compromised (Photo 1). The work carried out in the Sudanian zone of Burkina Faso by Ouédraogo (2006), Rabiou *et al*. (2015) and in Togo by Segla *et al*. (2015) showed that the natural stands of *P. erinaceus* are dominated by individuals of large diameter. Young or small diameter individuals are very rare. Natural seedlings are very rare and survival after one or two dry seasons as well as that of young plantations are very low (Bationo *et al*., 2010; Duvall, 2008). The study of its regeneration by economic means such as vegetative propagation could then encourage the regeneration of the species and increase the possibilities of its exploitation and valorization in Burkina Faso and Niger and in many other forests of the Subregion (Ganaba *et al*., 2005, Sawadogo *et al*., 2004). With regards to vegetative propagation, several techniques have been applied to species with regenerative difficulties (Bellefontaine, 2005, Ouédraogo, 2007, Zouggari, 2008). The commonly used techniques include the induction of

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root sucking by section, the air layering, stem segment cutting and root segment cutting (Bellefontaine *et al*., 2015). The work carried out by Ouédraogo (2007) on the vegetative propagation of *P.erinaceus* in Burkina Faso showed that root and stem segments did not produce roots. The same tests performed by Rabiou (2016) with 3 repetitions in different periods of the year using auxins as phytohormone were not successful. The cuttings of the stem segments have budded and even produced branches which eventually fade away as the reserves contained in the cuttings are exhausted. However, previous studies (Cuny *et al*., 1997 and Bellefontaine, 2005) have shown that the species suckers. The main difficulties, encountered in setting up and monitoring air layering, are the choice of layering rods. Indeed, in Sahelian and Sudanian zones, where *P. erinaceus* stands are dominated by large individuals, it is often necessary to climb the tree or use a ladder to set up and monitor the air layers. In addition, a lower density of individuals of *P. erinaceus* has been recorded in natural formations (Rabiou *et al*., 2015, Ouédraogo, 2006). The detection of reasonable irrigation frequencies makes it possible to reduce the difficulties encountered in monitoring air layering especially in areas dominated by old and scattered *P.erinaceus* trees. These difficulties are a hindrance to the spread of air-layering techniques and their appropriation by the local populations. The objective of this study is to develop techniques for vegetative propagation of *P. erinaceus* by airlayering. Specifically, it aims at identifying reasonable watering frequencies, the positions of the air layers on the branch and the diameter of the stems required for layering that optimize the rate of rooting. This scientific information is necessary for the improvement and diffusion of low-cost vegetative propagation techniques of *P.erinaceus* by air layering.

Photo 1. Pterocarpuserinaceus tree completely pruned for forage

MATERIALS AND METHODS

The layering consists in producing plants by inducing the appearance of roots on the branches of a woody species. This is done by bringing the stems into contact with an appropriate substrate (soil, sawdust, etc.). When the branch or stem of a woody species is placed directly against the ground to induce the root system, it is called layering by coating. When the contact with the substrate is above ground, it is called air layering. The experimental design was set up on 10-year-old *P.erinaceus* plantations with an average diameter of 15.8 ± 4.2 cm and an average height of 10.7 ± 2.5 m. The study was conducted on the research station of the Institute of Environment and Agricultural Research of Saria in Burkina Faso located around 100 km from Ouagadougou on the road to Koudougou. The average annual rainfall is 841.3 ± 118.16 mm (Figure 1) and an average annual temperature of 24 ° C. The soil is mostly dominated by sand or clay types. Two trials were conducted during the rainy season from July to September. During the first trial carried out in 2013, the air layers were watered every 20 days and the second trial conducted in 2014, the air layers were watered every week. The objective of the variation of the watering frequencies is to highlight the reaction of the air layers to drought and to determine the frequency of watering required to optimize rooting rates.

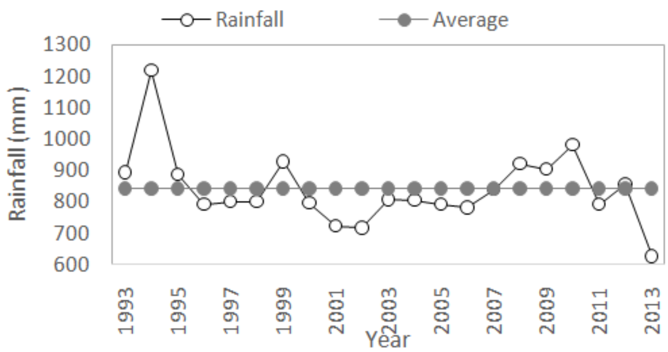

Figure 1. Rainfall variation at Saria station

Experimental design

A two-treatment device was set up for each trial. It is necessary to produce air layers with 3 different positions of the branch (apical, median and maximum) and for each position, 3 classes of diameter were considered (Table 1) in order to highlight the position and the Rod diameter required to optimize root production in number and length. The first trial carried out in 2013, where the air layers were irrigated every 20 days, encompassed 126 air layers distributed as follows;

- 18 air layers of the apical position with only one class of diameter (1-2 cm);
- 54 air layers of the median position with 3 classes of diameter (1-2; 2-3 and 3-4 cm) are 18 layers per class;
- 54 air layers of the proximal position with 3 classes of diameter (1-2, 2-3 and 3-4 cm) and 18 layers per class.

The second trial carried out in 2014, where the layers were watered weekly, is composed of 100 layers distributed as follows;

20 air layers of the apical position with a single class of diameter (1-2 cm);

- 40 air layers of the median position with 2 classes of diameter (1-2 and 2-3 cm) are 20 air layers per class;

- 40 air layers from the proximal position with 2 classes of diameter (1-2 and 2-3 cm) are 20 air layers per class.

Table 1. Summary of the experimental design and sampling of the layers

	Positions	10_20 mm	20_30 mm	30_40 mm
First trial (air layers under water stress)	Apical	18	-	-
	Median	18	18	18
	Proximal	18	18	18
Second trial (well-watered air layers)	Apical	20	-	-
	Median	20	20	-
	Proximal	20	20	-

Air layering technique

Beforehand, a mixture of soil and sawdust from the wood was prepared. The mixture is composed of 40% sawdust and 60% clay. For each layer, two annular incisions separated from each other by 5 cm in height were applied to the branch and without cutting the wood. This followed by a vertical incision of the bark starting from one annular incision to another. The portion of the bark is thus removed, leaving the wood bared (Photo 2A). The consequence is the interruption of the circulation of the sap elaborated from the upper part to the lower part of the incised stem. The diameter of the stem with and without bark is measured using a caliper to evaluate the thickness of the bark in function of the diameter of the branch.

Photo 2. Air layering of *P. erinaceus*; A: Stem Girdling; B: Air layersrealized

The stripped part of the bark is wrapped with a sheet of transparent plastic containing the mixture of soil and sawdust which serves as support for the roots. The two ends are then tightly sealed with scotch tape, in order to avoid trapping air. This substrate covers up to 10 cm on either side of the incised part. This sufficiently large substrate will allow a normal development of roots (Photo 2B). Water is supplied to the layers by injection with a 20 ml syringe. A quantity of 40 ml of water is injected for each watering. The holes caused by the introduction of the needle of the syringe are immediately closed with tape to minimize the evaporation of water. The trials were conducted during the rainy season, where photosynthetic activity and sap circulation were intense.

Data Collection and Processing

After each trial the layers were unpacked and cleared after 60 days of monitoring. For the second trial, 15 pits were weaned in the field for the viability test of the plants. The length of the longest root was measured with a ruler and the number of roots appeared was noted. The software R 2.15.3 was used for the analysis of the data. A test of X2 was used to test independence between the treatments, the rooting and mortality rates of the layers but also the number of roots that appeared per treatment. The general linear model (GLM) was used for performing the analysis of variance with two-factors for the comparison of lengths and average number of roots appearing by diameter class and position of the air layer. A logistic regression was used to test the significance of the rooting rates between the diameter classes.

RESULTS

Overall air layering success rate

The layers made for both trials were unpacked. Table 2 summarizes the overall rooting rates observed respectively for layers watered every week and those watered every 20 days. The results show that the overall mortality rate of air layers watered every 20 days is 46.8% and is of 44% for the watering grounds every week. Overall mortality rates are close for both trials. The highest total rooting rate was observed in the layers watered every week (56%) and lowest in the air layers watered every 20 days (19.8%). The healing call observed at the level of the air layers watered every 20 days is 33.3% whereas it is of 0% for the air layers watered every week. The results of the X2 test show the independence between the watering frequencies and the layering success rates (P<0,001).

Table 2. Overall air layering success rate

Air Layers	First trial		Second trial		Probability
	Number	%	Number	%	
Rooted	25	19,8	56	56	<0,001
Calogenesis	42	33,3	0	0	
Dead	59	46,8	44	44	
Total	126	100	100	100	

Success rate of air layering by position on the branch

When the air layers are watered every 20 days, the midline positioning air layers have the highest rooting rate (29.6%) followed by the layers of the apical position (22.2%). The lowest rooting rate (Photo 3A) was observed in the proximal

Table 3. Success rate of air layering by position on the branch

		Apical		Medians		Proximal		Probability
		Number	%	Number	%	Number	%	
First Trial	Rooted	4	22.2	16	29.6	5	9.3	P<0,001
	Calogenesis	7	38,9	24	44,4	11	20,4	
	Dead	7	38,9	14	25,5	38	70,4	
	Total	18	100	54	100	54	100	
Second Trial	Rooted	8	40	24	60	19	47,5	P=0,023
	Calogenesis	0	0	0	0	0	0	
	Dead	12	60	15	37,5	21	52,5	
	Total	20	100	40	100	40	100	

layers, resulting in a higher mortality rate in the latter (70.4%) (Table 3). The air layers watered every 20 days react by promoting the formation of callus of healing (clusters of undifferentiated cells) (Photo 3B). More than 44.4% of the median layers produced the healing callus instead of the roots.

When the air layers were watered weekly, more than 60% of the layers of the median position produced roots followed by the layers of the proximal position with 47.5% of rooting rate. The lowest rooting rate is observed at the level of the apical air layers. With regards to the air layers of the apical and proximal position, despite weekly watering, it is observed that the mortality rate is always higher than the rooting rate. This trend is reversed at the level of the air layers in the median position where the rooting rate is higher than the mortality rate. The tests of X2 confirm the independence between the positions of the layers on the branch on the one hand, and between the reactions of the layers to the watering frequencies on the other hand.

Rooting of the layers by position and by diameter class

The analysis of the interaction between the rooting rate of the air layers by diameter class and the position of the air layer on the branch shows that the layers of the proximal position of class of diameter between 1 and 2 cm present the highestrooting rate with 76.4% for the air layers watered every week and 33.3% for the air layers watered every 20 days.

However, the lowest rooting rate was recorded on the air layers of the apical position of diameter between 1 and 2 cm with 22.2% of the rootedair layers when watered every 20 days, compared to 23.5% for the air layers watered every week. The median positioning air layers were well distinguished in the second trial with rooting rates of 52.9% for the 1 to 2 cm diameter classes and 47% for the 2 to 3 cm classes (Table 4). The analysis of the results of the X2 test shows an independence between the rooting rates per diameter class and the position of the air layers on the branch with P = 0.047 for air layers watered every 20 days and P = 0.004 for air layers watered every week.

Table 4. Rootingrate of the interactions between diameter class and the position of the air layers

Trials	Positions	1_2cm	2_3cm	3_4cm	Probability
First Trial	Apical	22,2	-	-	0,047
	Median	27,7	33,3	27,7	
	Proximal	0	0	22,2	
Second Trial	Apical	23,5	-	-	0,004
	Median	52,9	47,0	-	
	Proximal	35,2	76,4	-	

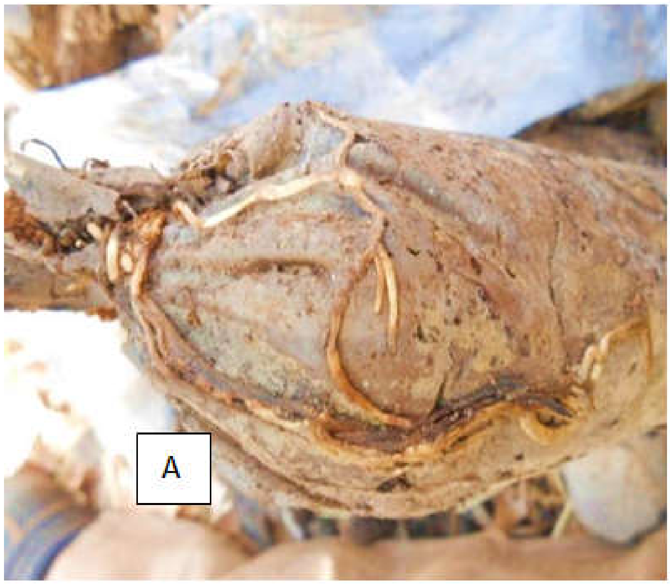

Photo 3. Rooted air layer (A) and formation of callus of healing (B)

Rooting by class of diameter of air layered stems

The diameter measurements carried out on the air layered branches allowed us to analyze the evolution of rooting rates as a function of diameter class. The analysis of the graph shows that the rooting rate is higher for the air layers with classes of diameter between 3 and 4 cm (53.8%) followed by the layers with a diameter ranging from 2 to 3 cm (44 , 7%). The lowest rooting rate was observed on the air layer of small-sized diameter (1 and 2 cm). In general, the rooting rate increases when the diameter increases (Figure 2). These observations were confirmed by the results of the logistic regression (P = 0.0054) (Table 6).

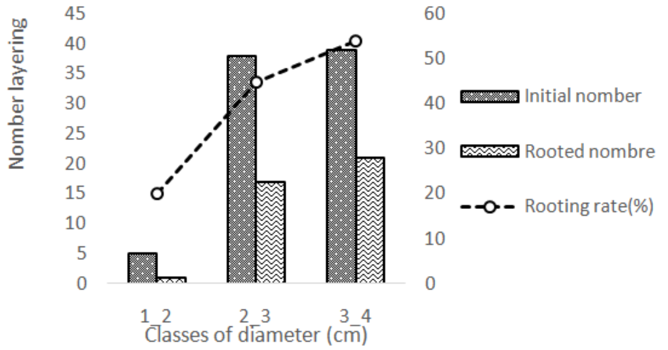

Figure 2. Evolution of rooting rate according to diameter classes

Characteristics of the air layered branches

The air layers that were set up, taking into account the variation of position and the diameter of the stems, allowed demonstrating the positions and the diameter ranges which maximize root production in terms of number and length. Furthermore, it has been shown that the diameter of the stems appears among the most important factors influencing the rooting rate. FIG. 4 illustrates the relationship between the diameter of the stem with bark and the thickness of the bark. Indeed, this relationship adjusts to a line of equation, thickness = 0,1626 Diameter - 0,5168. The coefficient of determination R2 shows that at the level of 73.8% the variation in the thickness of the bark is due to the variation in diameter of the stem with bark. It is noted that when the stem diameter increases, the thickness of the bark increases (Figure 3).

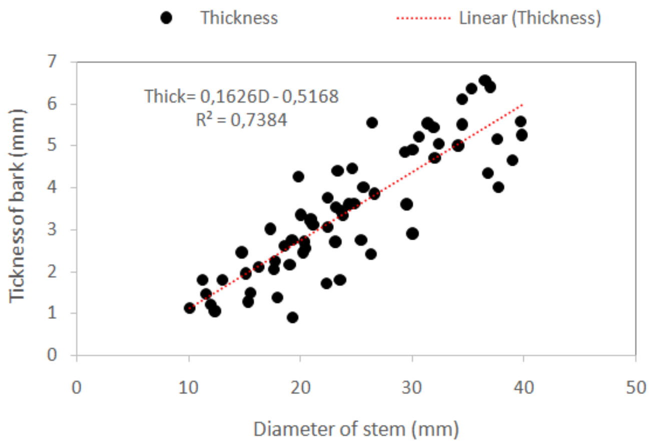

Figure 3. Relationship between stem diameter with bark and thickness of bark. D: stem diameter with bark; Thick: thickness of bark

Root structure appeared

The measurements of roots alloweddistinguishing the positions of the air layers and the stem diameter of the air layers, which optimize root production (Photo 4). The proximal air layers were well distinguished by the production of numerous roots (12.2 ± 9.6 roots) with an average length of 16.8 ± 3.9 cm (Table 5). The results are differentwhen considering the diameter classes of the branches. Indeed, the small-diameter air layers have the highest number of roots but of smaller length. The highest average of root length was recorded on the air layers with an intermediate class diameter (2 to 3 cm), followed by layers with a diameter ranging from 3 to 4 cm.

Table 5. Characteristics of appearing roots and measured after unpacking

		Mean Number	Mean Length (cm)
Positions	Apical	7,7a±10,9[a]	8,5a±1,9[a]
	Median	4,8b±4,5[b]	9,8±6,1[b]
	Proximal	12,2c±9,6[c]	16,8±3,9[c]
	Probability	0,005*	<0,001
Diameter of stem	1_2 cm	24,0a±4[a]	8,6±3,3[a]
	2_3 cm	6,3b±5,04[b]	15,4±9,9[b]
	3_4 cm	9,9c±10,16[c]	14,8±8,7[b]
	Probability	<0,001*	<0,001*

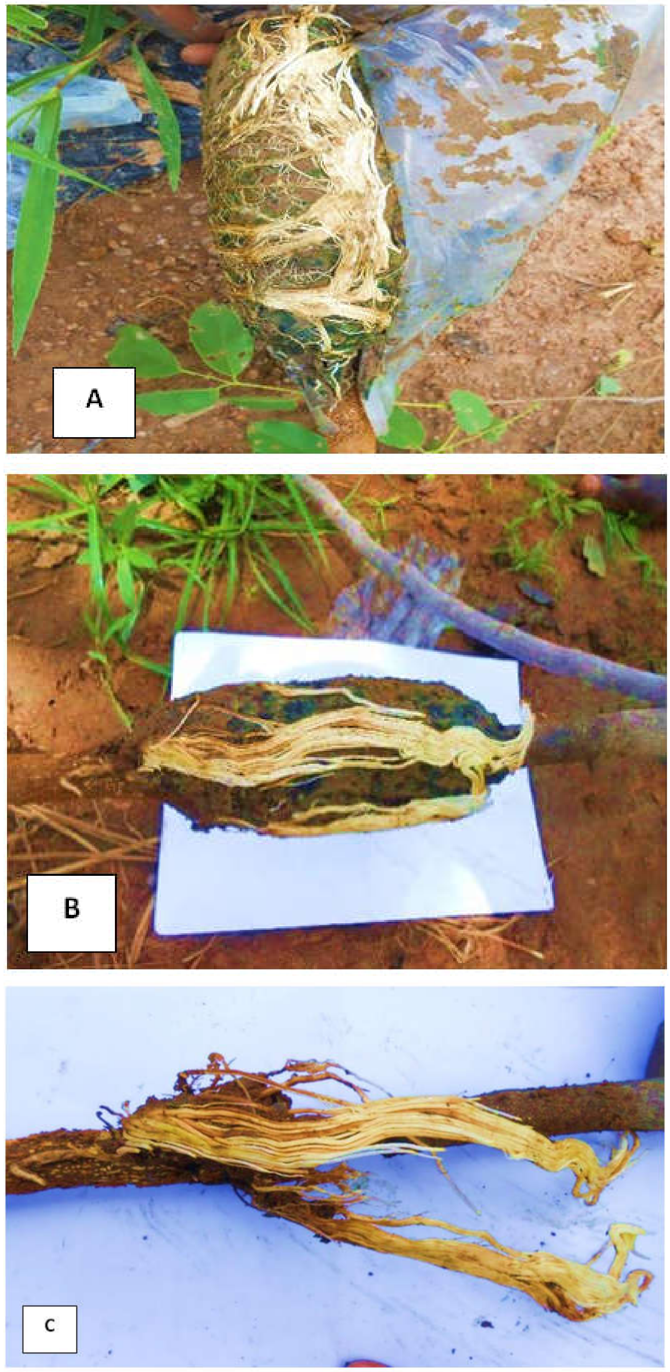

Photo 4. Rooted air layers, legend; A: Unpacked layers; B and C: rooted layers

The results of the analysis of the variances show significant differences in the number and length of the roots between the diameter classes and the positions of the layers on the branch ($P<0,05$) (Table 5).

Tableau 6. Results of logistic regression between rooting rate and diameter classes

Parameters	Estimate std.	Error	z value	Pr(>|z|)
(Intercept)	-0.2231	0.2535	-0.880	0.37881
Rate	0.9514	0.3424	2.779	0.00546**

DISCUSSION

Watering Requirement

The watering of layers once every 20 days allowed to more than 30% of the layers to develop callus of healing without being able to emit roots. However, when the layers are watered every week, none of them produced callus.The findings of this study show that calogenesis is therefore a response of air layers to water stress. These results also show that the layers must be regularly monitored and watered if necessary each week to ensure a permanent humidity of the substrate, which is essential for root development.

Aptitude of rooting of the layers

The rooting rate observed in the context of the present study is low (56%) compared to that observed by Ouédraogo (2007) on the strain rejection branches from P. erinaceus with a rate of 70%. This difference is explained by the difference in terms of viability conditions of the air layered stems.Indeed, the strain rejection from stems enjoys a supply by a well-developed root system with a higherrise of the raw sap. This higher ascent is also followed by the descent of a large amount of the elaborated sap that would be responsible for rhizogenesis. Another aspect related to the viability of the branches explaining the lower rooting rate in this study is the senescence of the lower branches in trees like P. erinaceus. Indeed, during its phases of growth, the tree lengthens its shaft by getting rid of the lower branches. The trialwas carried out on 10-year plantations. But at this age P. erinaceus is a growing tree. The lower branches undergo the senescence and eventually dry and disappear allowing the tree to lengthen its shaft. In this process of senescence the branch is no longer suitable for layering. Approximately 20% of the layers were placed in these branches andmost of them are part of the dead layers. The entire rooting rate observed in this study, although relatively lower compared to the findings ofOuédraogo (2007) on the same species, is higher to that observed by Laouali *et al*. (2015) on Prosopisafricana (Guill. and Perr.) Taub. with a rooting rate of 28.3%.

Analysis of the rooting rate of the layers showed that the medial and proximal layers located near the main stem of the tree are more suitable for layering with rooting rates of 60 and 47.5% respectively. Indeed, these positions are characterized by large diameters and thick barks. These two related characteristics play an important role in the distribution of the elaborated sap and the synthesis of the phytohormoneswhich is responsible for the cell differentiation. The analysis of the characteristics of the air layers allowedto demonstrating the strong relationship betweenthe diameter of the stem, the thickness of bark and the production of roots. The strong positive correlation ($R2 = 73.8\%$) observed shows that when the diameter of the stem increases, the thickness of the bark also increases. The thickness of the bark plays an important role in the storage of the elaborated sap and consequently the accumulation of the nutritive substances favorable to the cellular differentiation towards the rhizogenesis (Harivel *et al*., 2006).

The lowest layering ability was observed for the air layers from the apical position, i.e. at the extremity of the branch towards the terminal buds at a rate of 40%. This lower rooting rate can be explained by the characteristics of the branch. Indeed, layers at this position have branches of small diameter which does not support severe weather such as wind, drought, and the weight of the substrate. Many of them were quickly dried or broken by the wind before the end of the first month of monitoring. The rate of rooting depends on the position of the air layer on the branch. Zida (2009) observed a rooting rate of 72% at the proximal positions and 65% at the median positions in Balanitesaegyptiaca (L.) Del. and 40% at the median positions in Sclerocaryabirrea (A. Rich.) Hochst. but there was no reaction concerning the proximal layers. The evolution of this rate shows that the success of layering also depends on the position of the air layers on the branch. Indeed, proximal layers located near the main trunk of the tree have older woods compared to the apical and medial positions, and further have the highest average diameters and thicker bark capable of storing a large quantity of sap elaborated in its liber and supporting the rhizogenesis.

Evolution of number and length of roots

Analysis of the number of roots shows that there is no correlation between the age of the branch part and the number of roots that appeared. The number of roots appeared to be related to two factors which essentially are the proximity of the terminal buds, place of the synthesis of phytohormones responsible for cell differentiation, and proximity of the main trunk of the tree. This difference would be attributed to Phytohormones that regulate growth and stimulate rhizogenesis. In fact, auxin and cytokinin, which are stimulators of cell differentiation, are synthesized at the level of the buds, so that they are close to the apex of the apical position (Cornu and Boulay, 1986). This explains the large number of roots that have appeared on the level of the apical layers, because of their proximity to the buds, they maximize the accumulation of these hormones in favor of cell differentiation. With regards to the proximal layers, due to the size of their diameter and the thickness of their bark, they maximize the accumulation of the elaborated sap and sufficiently nourish the roots by promoting their growth. This explains the important length of the roots produced by air layers of the proximal position. The mechanism is such that large quantities of elaborate sap descending to the point of layering. The tissues at the site of the layering will be loaded with sap, which would stimulate the appearance of new roots (Bellefontaine *et al*., 2011).

Conclusion

Air layering of P. erinaceus in the present study on various branch positions, diameters and watering frequency resulted in obtaining transplantable seedlings in two months. The fifteenlayers, which belong to the second trial, weaned in the field and monitored, have resumed and all have provided viable individuals. The two trials carried out in 2013 and 2014 respectively on the air layers watered every 20 days and those watered every week allowed to highlight the watering frequencies, the positions and the diameter classes required for obtaining the best results. Indeed, the study showed that when the layers are watered every 20 days, they respond by producing calluses of healing, thus characterized by very few or without roots. To produce roots, the regular irrigation of layers is required once a week for maintaining the viability of the roots that appear. The medial and proximal positioning of the branch are those that optimize not only the success rate of layering but also maximize the appearance of long and numerous roots in P. erinaceus. These two positions on the branches of large diameter may contain a large quantity of the elaborated sap which is responsible ofrhizogenesis.

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