COMPARATIVE STUDY OF THE EFFECT OF RHIZOBACTERIA AND HORMONE ON TRECULIA AFRICANA (DECNE) CUTTINGS

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ABSTRACT

Treculia africana, a leguminous crop of the family Moraceace is widely grown and eaten in the South Eastern part of Nigeria. It serves economic, pharmaceutical and medicinal purposes. Plant growth promoting rhizobacteria (PGPR) are Plant rhizospheric bacteria which improves plant growth and yield via their various plant growth promoting substances. This study aimed at introducing PGPR (plant growth promoting rhizobacteria) as biostimulant during vegetative propagation. Cuttings of T. africana were inoculated using quick dip method, with 10^8cfu/ml of PGPR namely: *Pseudomonas fluorescens, Rhizobium larrymoorei, Streptomyces fumanus* and *Pseudomonas cissicola* isolated from rhizosphere of fruit trees using Pour Plate Method with serial dilution 10^2 on various selective medium. Growth parameters were taken after seven weeks under the humid propagator analysed using ANOVA followed by Duncan Multiple Range Test. The PGPR effects were then compared with hormone treated seedlings of T. africana. Results showed that, number of root initiated was significantly increased ($P<0.05$) by *Streptomyces fumanus* (13.00), followed by *Rhizobium larrymoorei* (10.75) (reduce to two decimals). The best hormone treatment on cutting roots was IBA 200 mg/l (9.25) while the control was 1.75. Number of shoot initiated was also significantly increased ($P<0.05$) by *Streptomyces fumanus* (6.75) followed by *Rhizobium larrymoorei* (5.00). The best hormone treatment on shoot was IBA 200 mg/l (3.00), while the control was 0.75. Also, PGPR treated cuttings were ready for pricking after the 7th week against the prevailing standard of 9 weeks.
The aforementioned PGPR should be considered as an alternative to synthetic hormones because their applications served as phytostimulant during the initiation of root and shoot of *T. africana*'s cuttings.

Keywords: *Treculia africana*, PGPR, Synthetic Hormones, Vegetative propagation

Accepted Date:23 July 2017

INTRODUCTION

Treculia africana Decne (African breadfruit) is an underutilised tree crop in the family Moraceae, it grows in the rainforest zone, particularly the swamp zone. It is widely grown in Southern Nigeria and North of the Democratic Republic of the Congo for its seeds and known by various tribal names in Nigeria such as Afon (Yoruba), Barafuta (Hausa), Ize (Bini), Eyo (Igala), Ediang (Efik) and Ukwa (Igbo) (Irvine, 1981; Onweluzo and Odume, 2008) and popularly known as Boimbo (Mongo) in the Democratic Republic of the Congo. The species is a large tree which grows up to 30m high, fruit collection is possible throughout the year with period of heavy fruiting between February and August alternating with that of light fruiting between September and January (Okafor 1985, cited in Nzekwe, Ojeifor, and Nworie 2010).

Treculia africana is increasingly becoming commercially important in Africa due to the potential use of its seeds, leaves, timber, roots and bark. It constitutes a cheap source of vitamins, minerals, proteins, carbohydrate, fats and is non poisonous (Osabor *et. al*., 2009). It is made into flour to be used: as soup thickener, Imitation milk, in bakery and Pharmaceutical industry. It provides

fodder for animals and the wood is put into various uses. It also has various medicinal uses. Yet, it was enlisted as high valued endangered indigenous fruit tree that needs to be domesticated (Nuga and Of odili, 2010 and Meregini, 2005).

It is therefore necessary to enhance its growth vegetatively using sustainable bio-resource technique such as PGPR. Positive outcome of this technique on cuttings will serve as a potential way to increase growth and yield of crops through vegetative propagation with little or no harm to the ecosystem as confirmed by Zakry *et al*., 2015 on enhancement of root of pepper stem cuttings by *Bacillus cereus* and Sekar and Kandavel, 2010 on growth performance of medicinal plants. PGPRs are group of rhizosphere colonizing bacteria that produces phytohormones, siderophores, antibiotics, Solubilize phosphate, inhibit plant ethylene synthesis, fixes Nitrogen and induce plant systemic resistances to pathogens (Saharan and Nehra, 2011 and Bhattacharyy, 2012).

The major objective of this study is to evaluate the effect of single and dual inoculation of *Pseudomonas fluorescens, Rhizobium larrymoorei, Streptomyces fumanus* and *Pseudomonas cissicola* on the initiation of new roots and shoots on *T. africana* stem cuttings in order to discourage the usage of synthetic hormones.

MATERIALS AND METHODS

This study was conducted in the fruit tree nursery of forestry Research Institute of Nigeria (FRIN) Ibadan Oyo state. FRIN is located on latitude 7^0 26' N and longitude 3^0 54'E of the equator. The climate of the area is tropically dominated by rainfall pattern between 1300 mm-1500 mm. The average temperature is about 36^0C and relative humidity ranges between 80-85% (Forest Conservation Unit, Forestry Research Institute of Nigeria, FRIN)

ISOLATION AND IDENTIFICATION OF PGPR

The PGPRs used for this study were isolated from soil samples collected from fruit trees rhizosphere in the Physiology fruit tree nursery of the Forestry Research Institute of Nigeria (FRIN) Ibadan Oyo state, using nutrient agar and various selective media and molecularly identified at International Institute of Tropical Agriculture (IITA) Ibadan Oyo state. They were confirmed to be Nitrogen fixing bacteria by subculturing on Burk's N-free medium while their plant growth promoting traits were ascertained by confirming their ability to solubilize Phosphate, Produce Indoleacetic Acid (IAA), Hydrogen Cyanide (HCN) and Ammonia.

INOCULATION OF CUTTINGS WITH PGPR AND SYNTHETIC HORMONE

Efficiency of PGPR inoculation on root formation of two-node stem cuttings of *T. africana,* was evaluated under nursery conditions in the Physiology fruit tree nursery of FRIN. The cuttings were treated with 10^8cfu/ml of the PGPR namely: *Pseudomonas fluorescens, Rhizobium larrymoorei, Streptomyces fumanus* and *Pseudomonas cissicola* and their possible combination respectively using quick dip method (Erturk *et al*., 2011). Distilled water served as the Control. Another set of two node stem cuttings of *T. africana* were also treated by dipping in solutions of IBA and NAA in the order below

- IBA---------- 150 mg/l, 200 mg/l and 250 mg/l
- NAA---------- 150 mg/l, 200 mg/l and 250 mg/l
- IBA/NAA---- 150 mg/l, 200 mg/l and 250 mg/l

The PGPR and hormone treated cuttings were then set in germination tray filled with sterilized river sand. Five cuttings per tray replicated four times and placed under humid propagator according to Sumbele, (2012) in a completely randomized design to develop new roots and shoots

The following growth parameters were assessed after the 7^{th} week.

- Number and percentage of cuttings that died by counting
- Number and percentage of rooted cuttings by counting
- Number and percentage of cuttings that callused by counting
- Number of roots by counting
- Length of roots using Meter ruler
- Number of shoots by counting
- Length of shoot using Meter rule
- Shoot diameter using digital Calliper

Data collected were statistically analysed using ANOVA. Means that were significantly difference at 5% probability level were separated using Duncan Multiple Range Test.

RESULTS

The plant growth promoting trait of the identified isolates showed on table 1. confirmed the PGPR used in this study as biofertilizers and phytostimulants. The effects of single and co-inoculation of the PGPR namely: *Pseudomonas fluorescens, Rhizobium larrymoorei, Streptomyces fumanus* and *Pseudomonas cissicola* on the growth parameters of *T. africana* cuttings are shown in the Table 2. The stimulatory effect of the PGPR on number of roots of *T. africana* stem cuttings (Table 2) was higher on cuttings treated with *Streptomyces fumanus* (13.00) followed by *Rhizobium larrymoorei* (10.75) while the control was 1.75 and the best hormone treatment was IBA 200 (9.25). The maximum root length was obtained in PGPR treatment *Streptomyces fumanus* (6.55cm) followed by *Rhizobium larrymoorei* (5.53cm) while the control was 1.8500 and the best hormone treatment was IBA 150 (3.33cm). The PGPR treatment, that most significantly affected shoot initiation was *Streptomyces fumanus* (6.75) followed by *Rhizobium larrymoorei* (5.00), the control mean number of shoot was 0.75 (reduce to two decimals) while the best hormone treatment was IBA 200 (3.00). In terms of shoot length hormone treatments IBA 200 (5.48cm), IBA 250 (4.35cm) and IBA150 (3.90cm) were better than PGPR treatments but among the PGPRs, treatment *Streptomyces fumanus* has the highest mean value (3.28cm) while the control mean value was 0.73cm. Table 2 further revealed that, the treatment with the highest mean of shoot diameter was *Rhizobium larrymoorei* (2.48mm) followed by *Streptomyces fumanus* (2.37mm), the control was 2.17mm while the best hormone was IBA 200 (2.04mm).

Cuttings number of leaves was significantly higher in cuttings treated with *Streptomyces fumanus* (10.50) followed by *Rhizobium larrymoorei* (9.50) and *Pseudomonas cissicola (9.50)*. The control mean value was 3.25 while the best hormone was IBA 200 (5.50).

The treatment with the lowest mortality rate was *Streptomyces fumanus* (1.00) indicating that cuttings treated with *Streptomyces fumanus* has the highest survival rate. IBA 150 and 200 has the lowest mortality rate (2.75) among the hormone treatments while the control has a mortality rate of 4.25 (Table 3).

Table 1: Qualitative analysis of plant growth promoting traits of the Rhizobacteria

Code	Selected PGPR	PS	NIT	HCN	NH_3	IAA
A	*Pseudomonas fluorescens*	+4	+	-	+	+
B	*Rhizobium larrymoorei*	+6	+	-	+	+
C	*Streptomyces fumanus*	-	+	-	+	+
D	*Pseudomonas cissicola*	+2	+	-	+	+

Keys:

PS-------Phosphate solubilization

NIT-----Nitrogen fixation

HCN----Production of Hydrogen cyanide

NH_3.......Production of ammonia

IAA-----Production of Indoleacetic Acid

Table 2: Mean performance of PGPR and Hormones on growth parameters of *Treculia africana*

Treatment	Number of root	Root length	Number of Shoot	Shoot length	Shoot diameter	Number of leaves
A	1.75^{cde}	2.50^{de}	2.75^{bcdef}	2.93^{bcde}	2.09^{abc}	5.75^{bcd}
AB	3.25^{bcde}	2.48^{de}	2.75^{bcdef}	1.75^{cde}	0.97^{cde}	4.50^{cde}
AC	9.25^{abcd}	3.18^{bcd}	3.25^{bcde}	2.38^{bcde}	1.83^{abcd}	7.75^{abc}
AD	3.75^{bcde}	1.30^{de}	2.00^{cdef}	1.58^{cde}	1.67^{abcde}	3.75^{cde}
ALL	0.50^{e}	1.10^{de}	2.75^{bcdef}	1.18^{de}	1.47^{abcde}	4.25^{cde}
B	10.75^{ab}	5.53^{ab}	5.00^{ab}	3.25^{abcde}	2.48^{a}	9.50^{ab}
BC	10.25^{ab}	5.23^{abc}	3.75^{bcd}	2.53^{bcde}	2.27^{abc}	7.50^{abc}
BD	0.00^{e}	0.00^{e}	1.00^{ef}	0.75^{de}	1.01^{cde}	1.00^{de}
C	13.00^{a}	6.55^{a}	6.75^{a}	3.28^{abcd}	2.37^{ab}	10.50^{a}
CONTROL	1.75^{cde}	1.85^{de}	0.75^{ef}	0.73^{de}	2.17^{abc}	3.25^{cde}
CD	2.00^{cde}	1.63^{de}	2.25^{cdef}	1.05^{de}	1.52^{abcde}	3.50^{cde}
D	10.00^{abc}	0.73^{de}	4.00^{bc}	3.00^{abcde}	1.99^{abc}	9.50^{ab}
IBA/NAA150	1.00^{de}	1.78^{de}	1.75^{cdef}	1.98^{bcde}	0.96^{cde}	4.00^{cde}
IBA/NAA200	0.25^{e}	0.00^{e}	0.25f	0.70^{de}	0.44^{e}	0.25^{e}
[illegible]	[illegible]^{e}	[illegible]^{de}	[illegible]^{def}	[illegible]^{cde}	[illegible]^{cde}	[illegible]^{de}
[illegible]	[illegible]^{abcde}	[illegible]^{bcd}	[illegible]^{bcde}	[illegible]^{abc}	[illegible]^{abcde}	5.00^{bcde}
[illegible]	[illegible]^{abcd}	[illegible]^{cde}	[illegible]^{bcde}	[illegible]^{a}	[illegible]^{abc}	[illegible]^{bcd}
[illegible]	[illegible]^{abcde}	[illegible]^{de}	[illegible]^{bcdef}	[illegible]^{ab}	[illegible]^{abcd}	[illegible]^{cde}
[illegible]	[illegible]^{e}	[illegible]^{e}	[illegible]^{ef}	[illegible]^{e}	[illegible]^{de}	[illegible]^{de}
[illegible]	[illegible]^{cde}	[illegible]^{de}	[illegible]^{def}	[illegible]^{cde}	[illegible]^{abcde}	[illegible]^{de}
[illegible]	[illegible]^{de}	[illegible]^{de}	[illegible]^{ef}	[illegible]^{cde}	1.08^{bcde}	[illegible]^{de}

Means in a column bearing the same letter are not significant at $p>0.05$. using Duncan's Multiple Range Test

Keys:

A--*Pseudomonas fluorescens*

B--Rhizobium larrymoorei

C--*Streptomycesfumanus*

NAA--Napthaleneacetic acid

IBA----Indolebutyric acid

D----Pseudomonas cissicola

EFFECT		SS	DF	MS	F	ProbF
Number of root	TRT	1510.738095	20	75.5369	3.077656	0.000353*
	Residual	1546.25	63	24.54365		
	Total	3056.988095	83	36.83118		
Length of root	TRT	266.307381	20	13.31537	4.616205	1.59E-06*
	Residual	181.7225	63	2.884484		
	Total	448.029881	83	5.39795		
Number of Shoot	TRT	196.1428571	20	9.807143	4.210221	6.23E-06*
	Residual	146.75	63	2.329365		
	Total	342.8928571	83	4.131239		
Length of Shoot	TRT	140.8657143	20	7.043286	2.920986	0.000632*
	Residual	151.91	63	2.41127		
	Total	292.7757143	83	3.527418		
Shoot Diameter	TRT	28.86972857	20	1.443486	2.287958	0.006737*
	Residual	39.747075	63	0.630906		
	Total	68.61680357	83	0.826708		
Number of leave	TRT	721.1428571	20	36.05714	4.473855	2.55E-06*
	Residual	507.75	63	8.059524		
	Total	1228.892857	83	14.80594		

Table 3: Mortality rate of PGPR, Hormone and the control rooted cuttings of *T. africana*

Treatment	Mortaliy
A- *Pseudomonas fluorescens*	3.00^{bcde}
AB- *Pseudomonas fluorescens / Rhizobium larrymoorei*	2.75^{cde}
AC-*Pseudomonas fluorescens / Streptomyces fumanus*	2.00^{def}
AD-*Pseudomonas fluorescens / Pseudomonas cissicola*	3.00^{bcde}
ALL- Combination of the PGPR	3.50^{abcd}
B- *Rhizobium larrymoorei*	2.00^{def}
BC- *Rhizobium larrymoorei / Streptomyces fumanus*	1.75^{ef}
BD- *Pseudomonas fluorescens / Pseudomonas cissicola*	4.50^{ab}
C- *Streptomyces fumanus*	1.00^{f}
Control	4.25^{abc}
CD- *Streptomyces fumanus / Pseudomonas cissicola*	2.75^{cde}
D- *Pseudomonas cissicola*	2.00^{def}
IBA/NAA 250 mg/l -Indolebutyric acid/ Napthaleneacetic acid	4.50^{ab}
IBA/NAA150 mg/l -Indolebutyric acid/ Napthaleneacetic acid	4.75^{a}
IBA/NAA200 mg/l -Indolebutyric acid/ Napthaleneacetic acid	4.25^{abc}
IBA150 mg/l -Indolebutyric acid	2.75^{cde}
IBA200 mg/l -Indolebutyric acid	2.75^{cde}
IBA250 mg/l -Indolebutyric acid	3.00^{bcde}
NAA150 mg/l-Napthaleneacetic acid	4.50^{ab}
NAA200 mg/l-Napthaleneacetic acid	4.25^{abc}
NAA250 mg/l- Napthaleneacetic acid	4.25^{abc}

Anova Table for Treatment Mortality Rate (Table 3)

EFFECT	SS	DF	MS	F	ProbF	
TRT	96.64285714	20	4.832143	5.690187	5.28E-08	**
Residual	53.5	63	0.849206			
Total	150.1428571	83	1.80895			

DISCUSSION AND CONCLUSION

Plant growth-promoting Rhizobacteria (PGPR) are commonly introduced to crops through seed and soil, inoculation of PGPR on stem cuttings is a less common practice. In this study, the four PGPR used were found to initiate roots and shoots of T. africana cuttings at 7 weeks against prevailing standard of 9 weeks when treated with synthetic hormone.

Results (revealed) that PGPR can enhance growth performance of *T. africana* cuttings better than hormone. All the four PGPR used showed stimulatory effect on the cuttings root number, root length, number of shoot and number of leaves. However, *Streptomyces fumanus* followed by *Rhizobium larrymoorei* were confirmed to improve shoot and root initiation of *T. africana* cuttings better than the other PGPR, Control and hormone treatments used. This showed that PGPR are species specific just like the synthetic hormone, thus, to get the maximum performance the right PGPR must be used.

This result is in agreement with the findings of Kang *et al.*, (2009) in which he reported the improvement of plant growth by some phytohormone producing PGPR. The growth performance of *T. africana* cuttings by *Streptomyces fumanus* in this study was also in comformity with Tinatin *et al*., (2015), in which growth of Wheat and Soybean were better improved when treated with *Streptomyces fumanus*. The increase in growth was attributed to growth stimulating compounds produced such as Auxin and Cytokinins which could enhance cell division, differentiation of root system and increase the number of lateral root hairs, consequently increasing the nutritional and respiratory surfaces of the root system as a whole. The healthier the root hairs, the more intensely they emit exudates and the more conducive the rhizosphere will be for root colonization by plant growth promoting rhizobacteria present in the rhizosphere.

Conclusively this study has been able to prove that, the use of single inoculation of the PGPR *Streptomyces fumanus* and *Rhizobium larrymoorei* can significantly increase the initiation of new root and shoot of the cuttings better than the hormone. The PGPR cuttings were ready for transplanting on the 7th week against the normal practice of 9 weeks as stated by Chinaka, (1998) and Sumbele, (2012). This confirms the possibility of replacing synthetic hormone with biofertilizers, which in turn will eradicate the problem of concentration of synthetic hormone to be used, problems associated with their preparation and applications.

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IN-VITRO PROPAGATION OF MANIHOT ESCULENTA CRANTZ THROUGH NODAL CULTURES

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ABSTRACT

Murashige and Skoog (MS) medium was supplemented with Naphthalene Acetic Acid (NAA) *and 6-benzylaminopurine BAP) in varied concentrations to determine the optimum concentration of the plant hormones needed for in vitro nodal proliferations and elongation of cassava plantlets.* Regenerated cassava nodal plantlets were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State. The nodal plantlets were excised and sub-cultured into different test-tubes containing MS medium supplemented with NAA and BAP at different concentrations of 0.005ml NAA + 0.02ml BAP, 0.01ml NAA + 0.05ml BAP, 0.015ml NAA + 0.08ml BAP and a control without any hormone for nodal proliferations and plantlet height. The results obtained showed that the control experiment not supplemented with hormones had the highest average number of nodes (40.33) at 9 weeks, while the MS supplemented with hormones at 0.01ml NAA + 0.05ml BAP yielded the longest plantlet (23.30mm) at 9 weeks. This study revealed that hormones in varied concentrations are necessary for early germination and elongation of cassava plantlets height but nodal proliferation of the cassava plantlets is not dependent on hormone supplements.

Keywords: Cassava, Murashige and Skoog, propagation, *in vitro* proliferation, plantlets.

Accepted Date: 4 August 2017

INTRODUCTION

Cassava is one of the crops in the tropical humid environments that is of great importance because of its availability as staple food, ease of cultivation, ability to change into different forms and stored as food for several years (Nassar *et al*., 2009; Le *et al*., 2007). It ranks fourth as a source of energy after rice, maize and sugarcane and it is a valuable source of calories especially in countries where malnutrition is widely spread (Chavez *et al*., 2000). It is predominantly grown by subsistence and commercial farmers in the sub-Saharan countries of Africa like Nigeria, Ghana, Burundi, Democratic Republic of Congo, Cameroon, Congo and host of others (Oyewole and Ogundele, 2001). Cassava improvement for increased production necessitates addressing various factors that beset its production. These factors include pests and diseases, genetics and breeding programs (Nassar and Ortiz, 2007) but more recently, it has been observed that micro-propagation has become an irreplaceable tool in the improvement and genetic manipulation of plant, especially vegetative propagated crops (Onuochi and Onwubiku, 2007). The ability to culture organized tissue in the form of very small shoots or meristem has allowed the most valuable application of plant tissue culture (Davies, 1981).

The techniques involved in Plant tissue culture were developed five decades ago (Santana *et al*., 2009) and since have been known to be powerful tools for studying and solving problems of production of cassava like many other crops