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EFFECT OF RHIZOBACTERIUM PSEUDOMONA SPECIES ON ROOT FORMATION OF *Treculia africana* DCENE (AFRICA BREADFRUIT) STEM CUTTINGS

[1]Odozie, E.C., [2]Amusa, N.A., [1]Oyedeji, O.F, [1]Adebusuyi, G.A., [1]Oladejo, S.F

[1]Forestry Research Institute of Nigeria, P.M.B. 5054, Jericho Hill, Ibadan, Nigeria

[2]Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria

Corresponding Email: edithodozie9@gmail.com Tel: 08033572902

Abstract

Rhizobacteria have been widely reported to be able to promote growth and yield of various agricultural crops through their various plant growth promoting trait. *Pseudomonas cissicola and Pseudomonas corrugate* were tested on their ability to induce and elongate roots of Africa breadfruit stem cuttings on 49th day of inoculation against the prevailing standard of 63rd day. Results showed the mean of rooted cuttings to be higher in hormone treatments IBA 150 mg/l and IBA 200 mg/l used (2) followed by *Pseudomonas cissicola* / *Pseudomonas corrugate (1.75)* over the control (0.75) but co-inoculation of *Pseudomonas cissicola*/ *Pseudomonas corrugate* (12.5) on Africa breadfruit stem cuttings stimulated number of roots on cuttings better than IBA 200 mg/l which was the best hormone treatment (9.25)and the control (1.75).Treatment with the longest root was co-inoculation of *Pseudomonas cissicola*/ *Pseudomonas corrugate* (5.78 cm) over the best hormone treatment IBA150 mg/l (3.325cm) and control (1.85cm).Present study recommends co-inoculation of *Pseudomonas cissicola*/ *Pseudomonas corrugate*as a potential candidate in a formulation of a biostimulant for organic and sustainable nursery for Africa breadfruit production. This practice can also be extended to other fruit tree species.

Keywords: Inoculation, Africa breadfruit, rhizobacteria, root induction, stem cutting

Introduction

African breadfruit (*Treculia africana* var. Africana Decne) multipurpose tree species also known as wild Jackfruit or African boxwood belongs to the family Moraceae and grows in the forest zone, particularly the coastal swamp zone (Agbogidi and Onomeregbor, 2008). Other members of the Moraceae family of plants that are used for food are *Artocarpus communis* and *Artocarpus heterophylla*. The sterile variety of the *A. communis* is the breadfruit, while the fertile variety with achene (usually called seeds) is the breadnut. The *A. heterophylla* is the jackfruit (Jean, 2014). African breadfruit is widely grown in Southern Nigeria for its seeds and known by various tribal names in the country, such names include Afon (Yoruba), Barafuta (Hausa), Ize (Bini), Eyo (Igala), Ediang (Efik) and Ukwa (Igbo) (Onweluzo and Odume, 2008). The most popular of these tribal names is Ukwa. It is commonly cooked, mashed and consumed as food with it liquid extract after cooking serving as juice among the elders or roasted and sold with palm kernel or coconut as roadside snack. It is also served as a special dish at ceremonies. Giami, *et al.* 2000 and Appiah, *et. al*., (2011) reported African breadfruit as a good source of imitation milk (vegetable milk) and a readily available, high quality alternative protein and energy.

Despite the dietary and economic importance of African breadfruit, it has remained an under-utilized species till now and its potentials remain under-exploited. Coupled with this problem, is the serious threat of erosion of its genetic resources as well as the extinction threat. The extinction threat on Africa breadfruit is increasing thereby threatened with severe genetic depletion (Sumbele, 2012) due to deforestation, non improvement and non cultivation of the species.

African breadfruits currently included in the list of endangered species of Southern Nigeria (Meregini, 2005) and this is quite worrisome. There is need to mass produce and improve these fruit trees species for domestication at sustainable level.

African breadfruit is conventionally propagated through seed but for mass production it is better propagated vegetatively. This technique is commonly practise due to cost effective and easy in planting material preparation. Hormones have been used and are still in use for cuttings root induction and they always produce excellent results (Sumbele, 2012) but the problem is the danger associated with inorganic farming. Plant growth-promoting rhizobacteria (PGPR) application can be propose as one of the alternative agricultural applications used to promote sustainability and health of plants. PGPR exhibit plant growth-promoting properties via several beneficial mechanisms such as phytohormone production, biological nitrogen fixation and phosphate solubilisation (Bhattacharyya, 2012). In the present study, the effects of inoculation of two species of Pseudomona namely: *Pseudomonas cissicola and Pseudomonas corrugate* inoculated as mono and co-inoculant on root formation of African breadfruit stem cuttings were evaluated under nursery conditions

Materials and Methods

The PGPRs used for this study were isolated from Soil sample collected from fruit trees rhizosphere in the physiology fruit tree nursery of the forestry research institute of Nigeria (FRIN) Ibadan Oyo state, located on latitude 7°23'15" to 7°24'00"N and longitude 3°51'00" to 3°52'15"E of the equator. The plant growth promoting rhizobacteria were isolated using nutrient agar and Kings B medium and molecularly identified at IITA Ibadan Oyo state. They were confirmed to be nitrogen fixing bacteria by subculturing on Burk's N-free medium while there plant growth promoting trait were ascertained by confirming their ability to Solubilize of Phosphate, Produce Indoleacetic Acid (IAA), hydrogen cyanide (HCN) and Ammonia.

The rhizobacteria species were molecularly identified to be *Pseudomonas cissicola and Pseudomonas corrugate.* They were then used in the present study for root induction of African breadfruit cuttings.

Efficiency of PGPR *Pseudomonas cissicola and Pseudomonas corrugate inoculation* on root formation of two-node African breadfruit stem cuttings was evaluated under humid propagator for seven weeks. Five treatments (control, 200 mg/l indole-3-butyric acid (IBA), 150 mg/l IBA, *Pseudomonas cissicola and Pseudomonas corrugate*) were assigned in a completely randomised design (CRD) with 5 replicates cuttings per treatment. In the present study, 200 mg/l and 150 mg/l indole-3-butyric acid (IBA), served as positive controls.

African breadfruit stem cuttings were treated by dipping them into 10^8cfm/ml of the PGPR treatments according to Erturk *et al*., (2011). Similar procedures were also employed on stem cuttings treated with IBA while the uninoculated controls were dipped into the Distilled water (Sumbele 2010 and Jean 2014) and set in germination trays under humid propagation containing sterilised river sand. The plants were irrigated once a day. After 49 days, the following data were taken on all the cuttings: percentage survival, number of rooted cuttings, total number of root per cutting, length of longest root per cutting (cm) and total length of roots per cutting (cm). A cutting was considered rooted if a minimum of one root = 1 mm in length was present. Percentagesurvival, indicating the percentage of African breadfruit stem cuttings in a treatment group is alive (still fresh) for a given period of time (49 days observations). Data collected were subjected to analysis of variance (ANOVA) in CRD followed by Duncan multiple range test for variables that are significantly different at 5% probability level.

Results

In this study, both *Pseudomonas cissicola* and *Pseudomonas corrugate* were reported as an IAA producer in addition to their ability to fix nitrogen and solubilise phosphate and the availability of this auxin was expected to benefit the development of adventitious roots in the

cuttings, however overconcentration of IAA might inhibit rooting since, according to Jarvis (1986), root elongation requires low concentrations of IAA (Tables 1-5).

Table 1: PGPR trait

PGPR Trait	*Pseudomonas cissicola*	*Pseudomonas corrugata*
PS	+	+
NIT	+	+
NH3	+	+
IAA	+	+
HCN	-	-

Keys:

- ---------- Absent

+ ---------- Present

PS----------Phosphorus solubilisation

NIT----------Nitrogen fixation

IAA----------Indo -3-acetic acid production

HCN----------Hydrogen cyanide production

NH_3----------Ammonia production

Rooting responses of African breadfruit cuttings to PGPR and IBA applications is presented in Table 2, PGPR treatments *Pseudomonas cissicola* (60%) and co inoculation of *Pseudomonas cissicola* / *Pseudomonas corrugate* (55%) significantly affect percentage survival of cuttings better than the hormone treatment (45%) and control (15%) but hormone treatment (5) were shown to significantly affect the mean number of rooted cuttings than the PGPR treatments *Pseudomonas cissicola*/ *Pseudomonas corrugate* (1.75). However, PGPR treatments were seen to have higher number of rooted cuttings than the control (0.75). The effectiveness of *Pseudomonas cissicola* / *Pseudomonas corrugate* co-inoculation on inducing numbers of roots on cuttings were significantly higher (12.5) compared to the control (1.75) and the best hormone treatment IBA 200 mg/l (9.25) African breadfruit stem cuttings treated with *Pseudomonas cissicola*/ *Pseudomonas corrugate* showed significantly increased length of the longest root in each cutting with mean value as 5.78cm as compared to control at 1.85 cm and the best hormone, IBA 150 mg/l as 3.33 cm.

Table 2: Percentage survival of PGPR and hormone rooted cuttings in Ibadan

Treatments	%Mortality	%survival
Sterile Distil Water	85	15
IBA 150 mg/l	55	45
IBA 200 mg/l	55	45
Pseudomonas corrugate	50	50
Pseudomonas cissicola / *Pseudomonas corrugate*	45	55
Pseudomonas cissicola	40	60

Table 3: Mean value of rooted cuttings of IBA, NAA and PGPR treated cuttings of *T. africana*

Treatment	Mean
Distil water	0.75^{d}
Pseudomonas corrugate	1.25^{c}
Pseudomonas cissicola	1.75^{b}
Pseudomonas cissicola/ *Pseudomonas corrugate*	1.75^{b}
IBA 150 mg/l	2.00^{a}
IBA 200 mg/l	2.00^{a}

Mean values with the same letter(s) in the same column are not significant at P>0.05 using Duncan's Multiple Range Test (DMRT)

Table 4: Mean number of roots on cuttings of IBA, NAA and PGPR treated cuttings of *T. africana*

Treatment	Mean value
Distil water	1.75[e]
Pseudomonas corrugate	4.25[d]
IBA 150 mg/l	8.5[c]
IBA 200 mg/l	9.25[b]
Pseudomonas cissicola	10[b]
Pseudomonas cissicola/ *Pseudomonas corrugate*	12.5[a]

Mean with the same letter(s) in the same column are not significant at P>0.05 using Duncan's Multiple Range Test (DMRT)

Table 5: Mean of root length of IBA, NAA and PGPR treated cuttings of *T. africana*

Treatment	Mean
Pseudomonas cissicola	0.725[e]
Distil water	1.85[d]
Pseudomonas corrugate	2.1[c]
IBA 200 mg/l	2.65[b]
IBA 150 mg/l	3.325[b]
Pseudomonas cissicola/ *Pseudomonas corrugate*	5.775[a]

Mean with the same letter(s) in the same column are not significant at P>0.05 using Duncan's Multiple Range Test (DMRT)

Discussion

Plant growth-promoting rhizobacteria (PGPR) have been used in conjunction with the cultivation of many important agricultural crops. They are commonly introduced through seed and soil inoculation. In this regard, the inoculation with PGPR on stem cuttings is a less common practice. In the present study, the effects of inoculations of two different pseudomona specie having plant growth promoting traits, on the stem cuttings of african breadfruit (*T. africana*.) were evaluated on root induction 49 days after inoculation. The 49 days period of observation was considered sufficient for variation in this study, in root induction which recorded some rooting parameters such as total number of roots in each cutting and maximum length of roots. This was against the prevailing standard of 63days under hormone treatment according to chinaka (1998) and Sumbele, (2010). The successful

treatment application in the present study would benefit African breadfruit nursery growers and serve as a prelude for further study on other fruit tress species.

Present study found that inoculation of co-inoculant of PGPR *Pseudomonas cissicola*/ *Pseudomonas corrugate* promoted root induction and stimulation of African breadfruit (*T. africana*.) stem cuttings. The co- inoculation of PGPR inoculatant was seen to be better than when the PGPR were inoculated as mono inoculant, it was also better than the control and synthetic hormoe, indole-3-butyric acid (IBA), applied at IBA 200 mg/l and IBA 150 mg/l. Application of IBA at this concentration has been reported as optimum concentration of synthetic hormone in enhancing rooting and sprouting of African breadfruit stem cuttings (Sumbele, 2010). IBA is widely used in agriculture as a commercial stimulant of root induction in cuttings (Dobbelaere *et al*., 2003) since chemical structure is nearly identical with indole-3-acetic acid (IAA) (Strader and Bartel, 2011), this gives good comparison with microbial IAA (Ali *et al*., 2009) also Synthetic forms of auxin are available commercially in the form of Indolebutyric acid (IBA) and Napthaleneacetic acid (NAA),

The growth observed in the control experiment is an indication that the plant endogenous hormone are capable of inducing root but with the introduction of hormone stimulant cuttings will be better enhanced (Alpheus, 2010). Increase in root induction by PGPR over the control confirms that there was an increase in the concentration of auxin in the rooting media which probably exert positive effect on the cuttings such as increase in the rate of xylem and root development, control process of vegetative growth and lateral and adventitious root initiation (Ahemad and Kibert, 2013).

The outcome of this study shows that with the right PGPR treatment, cuttings can be pricked into polypots between 7 to 8 weeks; this was against the established fact, that cuttings of *T. africana* must be left under propagator for 9 weeks before they can be successfully pricked into polypots (Chinaka, 1998 and Sumbele, 2010). In this study both *Pseudomonas cissicola* and *Pseudomonas corrugate* were reported as an IAA producer in addition to their ability to fix nitrogen and solubilise phosphate and the availability of this auxin was expected to benefit the development of adventitious roots in the cuttings, however overconcentration of IAA might inhibit rooting since, according to Jarvis (1986), root elongation requires low concentrations of IAA. Significant respond recorded in this study is an indication that the aforementioned PGPR was not deleterious to African breadfruit cuttings. Just like synthetic hormones PGPR are also specie specific. Thus there is need to use the right PGPR for each plant or crop. In this study both *Pseudomonas cissicola* and *Pseudomonas corrugate* were reported as an IAA producer in addition to their ability to fix nitrogen and solubilise phosphate and the availability of this auxin was expected to benefit the development of adventitious roots in the cuttings, however overconcentration of IAA might inhibit rooting since, according to Jarvis (1986), root elongation requires low concentrations of IAA. Significant respond recorded in this study is an indication that the aforementioned PGPR was

not deleterious to African breadfruit cuttings just like synthetic hormones PGPR are also specie specific. Thus there is need to use the right PGPR for each plant or crop, bacterial auxin PGPR produced depends on the response and physiological development of the plant due to endogenous hormone levels of its host, which may vary according to the genotype and age of the plant (Ahmad *et al*., 2005) Adaptation of this technique will eradicate the problem of concentration of synthetic hormone to be used, problems associated with their preparation and application.

Thus it might be said that co-inoculation of *Pseudomonas cissicola* / *Pseudomonas corrugate* a plant growth promoting rhizobacteria used in this study with their multifunctional properties will attract more attention in the field of bio-fertiliser and phytostimulant. Also, it will play an important role toward achieving the objectives of sustainable agriculture.

Conclusion

Introduction of PGPR to Nigeria farming system will definitely meet the nation expectation in agricultural sector thus reducing the long list of dangers associated with agrochemical products. Therefore, production of *T. africana* seedlings by employing the right plant growth promoting rhizobacteria treatment(s), during vegetative propagation presents itself as a promising technology, given the results obtained in this study.

The aim of this study is to determine the possibility of raising good seedling of *T. africana* especially through vegetative propagation using PGPR that can effectively compete with synthetic hormones. This has been clearly achieved, as cuttings of African breadfruit responded positively to co-inoculation of plant growth promoting rhizobacteria, *Pseudomonas cissicola*/ *Pseudomonas corrugate* better than the synthetic hormone and the control.

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