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The Growth Enhancement Potentials of Indigenous Plant Growth Promoting Rhizobacteria on Sweet Pepper (*Caspicum annum*) through Seed Bacterization

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Abstract

The demand for food is rising as the world's population is rising. The second sustainable development goal's objectives of achieving global food security and agricultural sustainability call for swift and coordinated action. The use of traditional mineral fertilizers has increased dramatically in response to the rise in food demand, but this has its drawbacks.

Plant growth-promoting rhizobacteria (PGPR) which is a free-living bacteria actively colonize plant roots and aids it growth. They provide an environmentally friendly way to boost plant output. Due to the high nutritional content and wide range of medicinal applications of sweet pepper (*Capsicum annuum* L.), it constitute one of the most significant vegetable crops in the world.. sweet pepper (*Capsicum annum*) was evaluated through a pot study under the treatment of six bacterial strains. Bacterization of sweet pepper seeds was done via the immersion of seeds in a suspension of bacteria (10^8 CFU/ml) modified with 2% carboxymethyl cellulose (CMC), The treated seeds were planted in sterilized planting bags with sterilized sand, Effects of bacterized seeds of *Capsicum annuum* on the shoot height revealed that the highest shoot height was observed at week 8 by isolate AU4(*Burkholderia* sp) at (9.66±12.15), followed by AU6 (*Burkholderia* sp) at week 6
(6.26±0.05). The highest leaf number of *Capsicum annuum* after bacterization was observed at week 8(9.00±0.0) and 10(9.00±0.0), while the observed control at week 8(6.00±1.0) week 10(7.00±0.0). Plant growth-promoting bacteria applied via seed bacterization has shown potential for increasing the growth parameters of sweet pepper. This could be a formidable substitute for the use of chemical fertilizers. The use of *Burkholderia vietnamiensis* AU2011 and *Burkholderia* sp have shown potential for the increment of the agronomical parameter of *C. annuum*.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), Bacterization, Sweet Pepper, Seed.

1.Introduction

The world population is on the increase. As a consequence, leading to an increase in food demand. Several years ago, the millennium development goals (MDGs) were established to change the narrative of hunger in developing nations. A set of aspirational goals and targets that should direct every country's efforts in the pursuit of a better world were formed in 2015 by a global development agenda presented by the international community. All aspects of sustainability—social, economic, and environmental—are covered by the sustainable development goals (SDGs) [1], [2].

The second sustainable development goal's objectives of achieving global food security and agricultural sustainability call for urgent and coordinated action on the part of developing nations, where hunger is still a problem and sustainable agriculture is seldom ever practiced [2]. Sustainable Development Goal two borders on eradicating hunger, ensuring food security and nutrition, improving diets, and advancing sustainable agriculture. Due of its fundamental connections to society, the economy, and the environment, SDG-2 is crucial to achieving the whole SDG goal [3].

The Yoruba people of Nigeria's southwest have a proverb that goes, "If hunger is solved, man's biggest problem is solved." Due to the rapid growth in the world's population and the existing amplified food demand, the utilization of traditional mineral fertilizers has been rising drastically [4]. Synthetic fertilizers can boost crop growth and yields, but their broad use has detrimental side effects that include water pollution, pesticide reinforcement, soil salinization, and soil hardening [5]. As a result, there is a high demand for creating and using novel alternative inputs in agriculture.

Free-living bacteria, such as plant growth-promoting rhizobacteria (PGPR), colonize plant roots and have a beneficial impact on the growth of the plant by enhancing root development, increasing plant nutrient and water uptake, and boosting enzyme activity [6]. New sustainable vegetable production is moving toward techniques that mitigate stress events and increase quality and yield. Foliar fertilizers, biostimulants, and plant growth regulators, as well as recent innovative techniques like nanotechnology, could play a significant role in the improvement of agricultural products [7], [8].

Due to the nutritional content, therapeutic effect and its economic significance, sweet pepper (*Capsicum annuum* L.) is one of the most significant vegetable crops in the world [9].

Sweet pepper (*Capsicum annum*) is a perennial crop that is rich in minerals. It has higher protein content compared to cassava and yam but is low when compared to legumes [10]. This study seeks is to use microorganisms to promote the growth of sweet pepper through seed bacterization indicated by the measurement of the shoot height, the number of leaves, and leaf lengths. To the best of our knowledge, this is the first time indigenously isolated plant growth-promoting bacteria of the *Burkholderia* genera is used to enhance the growth of Sweet pepper (*c*) through bacterization in Nigeria.

We hypothesize that bacterization by indigenous plant growth-promoting rhizobacteria will enhance the growth and strongly contributes to the fitness and health of sweet pepper. Finding the indigenous PGPR's growth enhancement potentials may be essential for creating biologically based solutions for improved crop robustness and health, and subsequently for the long-term development of smallholder crop production systems in rural Africa. This study was aimed at increasing the agronomical parameters of sweet pepper by bacterization with PGPR.

2.Materials and Methods

Soil sample collection: Rhizosphere soil samples of soybeans (*Glycine max*) plant were collected from a farm in the Egbeda area of Ede, Osun State, Nigeria, with geographical coordinates 7.7604° N, 4.4615° E The rhizospheric soil was obtained by shaking method and subsequent brushing of remaining root system soil. The samples were carefully collected and transferred in sterile polythene bags and labeled properly [11], [12].

2.1Isolation and CFU Determination of Bacterial Strains

The isolation of bacterial was done in line with the protocol of [13]. Briefly, the soil sample (1g) was serially diluted by placing it in 9ml of distilled water and agitated for 15mins. This was followed by serially diluting each suspension from 10^{-1} to 10^{-6} in a bid to reduce the microbial load. After a series of dilutions, it was plated on a nutrient agar medium (NAM) in a spread plate manner where 0.1ml of the suspension was introduced to the surface of the agar and evenly distributed with a sterile glass rod. Incubation was done at 37°C for 24 hours. The pure culture of morphologically distinct colonies was obtained via successive subcultures and stored in the refrigerator for further analysis.

2.2Characterization of Rhizibacterial Isolates for Different Plant Growth Promoting Activities

2.2.1 Phosphate Solubilisation test was done with insoluble tricalcium phosphate (TCP), where fresh bacterial cultures were streaked on the center of Pikovskaya agar and incubated for 120 hours at 28±2°C. The constitutents for the pikovskaya agar were as follows: Dextrose 10.00 (g/l), calcium phosphate 5.00 (g/l), ammonium sulfate 0.50 (g/l), magnesium sulfate 0.10 (g/l ferrous sulfate 0.0001 (g/l), yeast extract 0.50 (g/l), potassium chloride 0.20 (g/l),), manganese sulfate 0.0001 (g/l). A halo zone surrounding the bacterial colonies showed that they were capable of solubilizing phosphate (Pande *et al*., 2017).

2.2.2 By inoculating a 250µL of cell suspension from each isolate into a 5 mL of nutritional broth containing tryptophan (0.1 g/L) and incubated at 30 °C for 7 days, the ability of the isolates to produce indole acetic acid was tested. The cultures were centrifuged for 10 minutes at 10,000 rpm. Using Salkowski's reagent, a 500 µL supernatant was combined with a drop of 1 mL of orthophosphoric acid (85%) , and incubated at room temperature for 15 minutes until pink (a sign of indole synthesis) [14].

2.2.3 Production of Siderophores, Bacteria samples were introduced and incubated for 24 hours at their preferred temperatures on Chrome Azurol S (CAS) agar. Blue to purple and blue to orange hue shifts were signs of the production of siderophore [14].

2.2.4 Production of hydrogen cyanide (HCN). Isolated bacteria grown on nutrient agar that contained 4.4 g/L of glycine. The agar was covered with Whatman No. 1 filter paper that had been dipped in a solution that contained 0.5% picric acid and 2% sodium carbonate by weight. Parafilm paper was used to seal the plates, which were then incubated at 36 ±2° C for 4 days. When red colour formed, orange indicated the presence of hydrogen cyanide [15].

2.3 The determination of the catalase activity of the isolate was done by adding Hydrogen peroxide to Nutrient agar grown samples. The production of oxygen as effervescence indicated the activity of the enzyme catalase.

Biochemical Test of including, Citrate, Gelatin hydrolysis, Gram staining, H_2S, Indoles, Nitrate Reduction, Oxidase, and Urease of the isolates was done to determine their presumptive identity.

2.4 Whole Genome Sequencing

The complete genome of AU1(*Enterobacter sichuanensis* AJI2411), AU4(*Burkholderia contaminans* OYA 0604), and AU5(*Burkholderia vietnamiensis* AU2011), were done. The bacteria's whole genome sequencing (WGS) was performed using genomic DNA isolated with a genomic DNA purification kit (Promega, USA). The Nextera DNA Flex Library preparation kit (Illumina, San Diego, CA, USA) was employed in the preparation of the libraries and then sequenced on the MiSeq sequencing platform using a 2 x 300 cycle V3 kit, following normal Illumina sequencing techniques (Illumina, San Diego, CA). The jekesa pipeline (https://github.com/stanikae/jekesa) was employed for bacterial WGS typing. Trim-galore v [16] was used to quality-filter paired-end reads. De novo assembly was performed using SKESA v2.3.0 [17] and optimized using shovill(https://github.com/tseemann/shovill).

Plant growth promotion assay and Experimental design: The growth of sweet pepper (*Capsicum annum*) was investigated through a pot study under the treatment of six bacterial strains. The experiment consists of a control group (unbacterized) and six different bacterial strains. Each treatment was replicated three times and arranged in a completely randomized block design. The experiment was conducted in 10″ inch planting bags filled with sterilized field soil (7 kg soil pot−1) in greenhouse conditions at the Microbiology Department of Adeleke University, Ede, Osun State, Nigeria.

The seeds of *Capsicum annum* were disinfected for 3 minutes with a 2.5% sodium hypochlorite solution, rinsed 3 times with sterile distilled water, and air-dried. After soaking for 30 minutes in a suspension of bacteria (10^8 CFU/ml) modified with 2% carboxymethyl cellulose (CMC), the seeds were air-dried for 12 hours. The treated seeds were planted in sterilized planting bags with sterilized sand, while the control was seeds treated with just 2% carboxymethyl cellulose (CMC). The planting bags were kept in an experimental greenhouse with a 12hour photoperiod, a temperature of 25^0C, and daily watering [18]. Plant growth-promoting rhizobacteria were established in earlier investigations for their ability to produce IAA, ACC deaminases, Phosphate solubilization, nitrogen fixation, ammonia, and HCN, was used for bacterization was obtained from the Department of Microbiology, Adeleke, University, Ede.

2.5 Statistical analysis: With SPSS software, one-way ANOVA was used to evaluate all of the sweet pepper growth parameter data that were collected from the plant growth promotion assay. The experiments were run in triplicate.

2.6 Measurements of Agronomical Parameters: The agronomical parameters for the *Capsicum annuum* were measured at weeks 2, 4, 6, and 8, while [19]. Plant Height, Number of Leaves per Plants [20].

3. RESULTS

After serial dilution, spread plating, and other basic microbiological basic methods, on the soil sample, a hundred and eight morphological distinct colonies were observed. Isolates were subjected to further screening, for plant growth-promoting activities. Table 1 is a highlight of the screening test of the six selected isolated plant growth-promoting rhizobacteria majorly populated by *Burkholderia* specie and Table 2 is a chronicle of the biochemical test performed. All isolates were negative for hydrogen sulphide reduction, Indoleand Urease.

Table 1: Screening for Plant Growth Promoting Rhizobacteria

IC	GR	S	IAA	SP	PS	NH_3	HP
AU 1	--ve	Rod	+	+	+	+	+
AU 2	-ve	Rod	+	+	-	-	-
AU 3	-ve	Rod	+	+	-	-	-
AU 4	-ve	Rod	+	+	+	+	+
AU 5	-ve	Rod	+	+	+	+	+
AU 6	**-ve**	**Rod**	**+**	**-**	**+**	**-**	**-**

Key: IC:Isolate Code, GR:Grams Reaction, S:Shape, IAA: IAA production, SP: Siderophore Production, PS: Phosphate Production, NH_3: Ammonia Production, HP: HCN Production

Table 2: Biochemical Test for Isolates Identity

S/N	IC	CS	CT	GT	NT	OX	Presumptive Identity
1	AU1	-	+	+	+	+	*Enterobacter*
2	AU2	+	+	+	+	+	*Burkholderia sp*
3	AU3	+	+	+	+	+	*Burkholderia sp*
4	AU4	+	+	+	+	+	*Burkholderia sp*
5	AU5	+	+	+	+	+	*Burkholderia sp*
6	**AU6**	+	+	**+**	+	+	*Burkholderia sp*

Key: IC: Isolate Code, CS: Catalase,CT:Citrate,GT:Gelatin hydrolysis, NT:Nitrte,OX:Oxisase + = positive, - = negative

3.1 Whole Genome Result of PGPR

Out of the isolates that displayed plant growth-promoting activities, three isolates were subjected to whole-genome sequencing and are presented in Tables 3,4, and 5 respectively. The features of the isolates containing the genes, RNA,and Pseudo genes amongst others are listed.

Table 3: Genome features of *Burkholderia contaminans OYA 0603*

Name	Burkholderia contaminans OYA 0603
Locus	NZ_JACWLL010000000 8589491 bp DNA linear BCT 30-SEP-2020
BioProject	PRJNA224116
BioSample	SAMN16073605
Assembly	GCF_014773305.1
Method of Assembly	SKESA version 2.3.0
Genome Representation	Full
Genome Coverage	61x
Sequencing Technology	Illumina MiSeq
Annotation Provider	NCBI RefSeq
Annotation Pipeline	NCBI Prokaryotic Genome Annotation Pipeline (PGAP)
Method of Annotation	Best-placed reference protein set; GeneMarkS-2+
Annotation Software revision	4.13
Genes (total)	7,928
CDSs (total)	7,856
Genes (coding)	7,695
CDSs (with protein)	7,695
Genes (RNA)	72

rRNAs	5, 1, 3 (5S, 16S, 23S)
rRNAs (complete)	5 (5S)
rRNAs (partial)	1, 3 (16S, 23S)
tRNAs	59
ncRNAs	4
Pseudo Genes (total)	161
CDSs (without protein)	161
Pseudo Genes (ambiguous residues)	0 of 161
Pseudo Genes (frameshifted)	34 of 161
Pseudo Genes (incomplete)	134 of 161
Pseudo Genes (internal stop)	9 of 161
Pseudo Genes (multiple problems)	14 of 161
WGS_SCAFLD	NZ_JACWLL010000001-NZ_JACWLL010000141

Table 4: Genome features of *Burkholderia vietnamiensis* AU2011

Name	Burkholderia vietnamiensis strain AU2011
Locus	NZ_CP072321 1335482 bp DNA circular CON 06-APR-2021
BioProject	PRJNA224116
BioSample	SAMN18299381
Assembly	GCF_017742755.1
Assembly Method	SKESA v. v2.3.0
Genome Representation	Full
Genome Coverage	14.8x
Sequencing Technology	Illumina MiSeq
Annotation Provider	NCBI RefSeq
Annotation Pipeline	NCBI Prokaryotic Genome Annotation Pipeline (PGAP)
Annotation Method	Best-placed reference protein set; GeneMarkS-2+
Annotation Software revision	5.1
Genes (total)	6,146
CDSs (total)	6,084
Genes (coding)	5,851
CDSs (with protein)	5,851
Genes (RNA)	62
rRNAs	4 (5S)
complete rRNAs	4 (5S)
tRNAs	54
ncRNAs	4
Pseudo Genes (total)	233
CDSs (without protein)	233
Pseudo Genes (ambiguous residues)	97 of 233
Pseudo Genes (frameshifted)	68 of 233
Pseudo Genes (incomplete)	113 of 233
Pseudo Genes (internal stop)	8 of 233
Pseudo Genes (multiple problems)	51 of 233
Accession	NZ_CP072321.1

Table 5: Genome features of *Enterobacter sichuanensis* AJI2411

Name	Enterobacter sichuanensis AJI2411
Locus	NZ_JACWFD010000000 5032127 bp DNA linear BCT 29-SEP-2020
BioProject	PRJNA224116
BioSample	SAMN16081429
Assembly	GCF_014779575.1
Method of Assembly	SKESA version. 2.3.0
Representation of Genome	Full
Genome Coverage	111x
The Technology of Sequencing	Illumina MiSeq
Annotation Provider	NCBI RefSeq
Pipeline ofAnnotation	Prokaryotic Genome Annotation Pipeline (PGAP) of NCBI
Annotation Method	Best-placed reference protein set; GeneMarkS-2+
Annotation Software revision	4.13
Genes (total)	4,943
CDSs (total)	4,852
Genes (coding)	4,766
CDSs (with protein)	4,766
Genes (RNA)	91
rRNAs	5, 1, 3 (5S, 16S, 23S)
complete rRNAs	5 (5S)
partial rRNAs	1, 3 (16S, 23S)
ncRNAs	77
Pseudo Genes (total)	86
Pseudo Genes (ambiguous residues)	0 of 86
Pseudo Genes (frameshifted)	34 of 86
Pseudo Genes (incomplete)	53 of 86
Pseudo Genes (internal stop)	17 of 86
Pseudo Genes (multiple problems)	16 of 86
WGS_SCAFLD	NZ_JACWFD010000001-NZ_JACWFD010000060

3.2 Measurement of Agronomical Parameters of Bacterized seeds

Effects of bacterized seeds of *Capsicum annuum* on the shoot height revealed that the highest shoot height was observed at week 8 by isolate AU4(*Burkholderia* sp)(9.66 ± 12.15^{a}); followed by AU6 (*Burkholderia* sp) at week 6 (6.26 ± 0.05^{a}). The highest leaf number of *Capsicum annuum* after bacterization was observed at weeks 8(9.00 ± 0.0^{a}) and 10(9.00 ± 0.0^{a}), while the observed control at weeks 8 and 10 were 6.00 ± 1.0^{bc} and 7.00 ± 0.0^{b} respectively. The highest observed leaf length is observed at week 10(6.15 ± 0.16^{a}) by isolate AU6. Error bars indicated standard deviations of three replicates (Figures 1, 2, and 3 below).

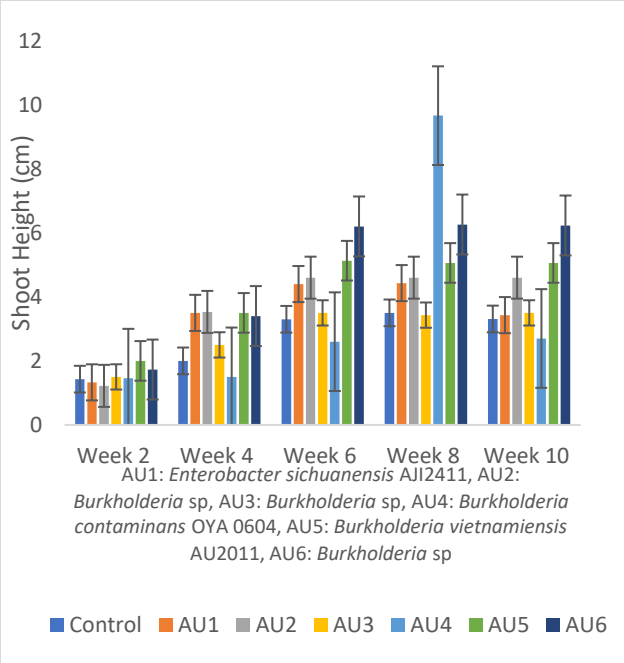

Figure 1: Effect of bacterization on Shoot height

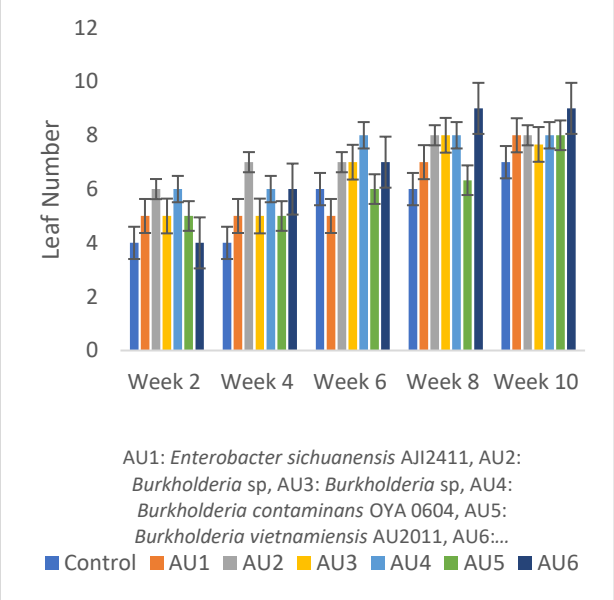

Figure 2: Effect of bacterization on Leaf Number

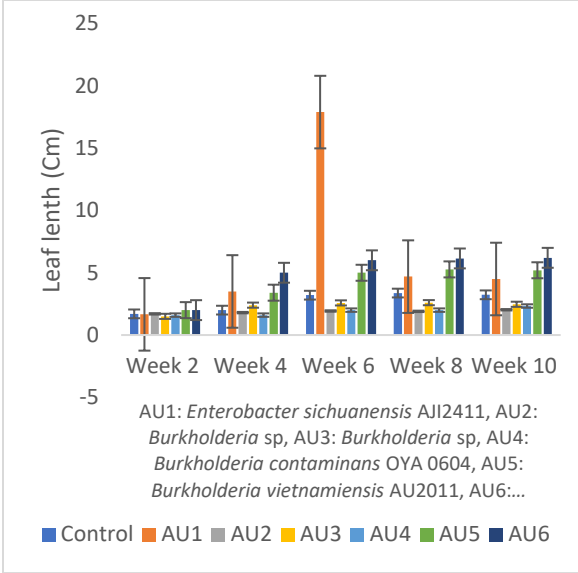

Figure 3: Effect of bacterization on Leaf Length

4. Discussions

Plant-growth promoting rhizobacteria are free-living organisms that actively colonize the roots of plants and have a positive impact on their growth. Plant growth-promoting microorganisms can stimulate plant growth and solubilize phosphates using their metabolism. They can directly impact plant metabolism by producing hormones and fixing nitrogen [21]. This is the first report on the growth enhancement of sweet pepper through the seed bacterization by Plant Growth Promoting Rhizobacteria (PGPR) to the best of the authors knowledge. In the present study, the leaf length of *C. annuum* at week 10 by *Burkholderia vietnamiensis* AU2011(5.20 ± 0.11^{b}), and *Burkholderia* specie AU6(6.20 ± 0.1^{a}) increased over the control that was (3.23 ± 0.35^{d}) as indicated in figure 3. The increase in leaf length is evidenced of growth. The increase in growth is in tandem with the study of [22], where the growth of *C. annuum* was enhanced using seaweeds. The leaves of sweet pepper are also used as ingredients in concoctions used by locals for the treatment of ailments. The leaves are also consumed. The shoot height of *C. annuum* increased significantly at week 8 by isolate AU4 (*Burkholderia contaminans* OYA 0603). Figure 3 showed an increase in leaf number by all the test isolates at weeks 8 and 10. This is in testament to the plant growth-promoting potential of the isolates

5. Conclusions

In our study, we found that the growth enhancement via seed bacterization is an environmentally sustainable opportunity to produce crops with the potential of replacing excessive fertilizer use. The identification of microbial isolates that are indigenous and adapted to Africa, will serve as potential plant growth enhancement for small-scale African farmers**.** Plant growth-promoting bacteria applied via seed bacterization has shown potential for increasing the growth parameters of sweet pepper. This could be a formidable substitute for the use of chemical fertilizers. The use of *Burkholderia vietnamiensis* AU2011 and *Burkholderia* sp have shown potential for the increment of the agronomical parameter of *C. annuum*.

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REFERENCE

[1]. (UN, 2015; UN. 2015. Transforming our world: The 2030 agenda for sustainable development. New York: United Nations, Department of Economic and Social Affairs.

[2]. Gil *et al*., 2019). Gil, J.D.B., Reidsma, P., Giller, K., Todman, L., Whitmore, A., & van Ittersum, M. (2019). Sustainable development goal 2: Improved targets and indicators for agriculture and food security. *Ambio*, 48,685–698

[3]. FAO 2016 FAO. 2016. Food and agriculture: Key to achieving the 2030 agenda for sustainable development. Rome: Food and Agriculture Organization.

[4]. (Benson *et al*., 2018) Benson, T., Lavelle, F., Bucher, T., McCloat, A., Mooney, E., Egan, B., Collins, C., & Dean, M.(2018).The Impact of Nutrition and Health Claims on Consumer Perceptions and Portion Size Selection: Results from a Nationally Representative Survey. Nutrients 20, 10(656), 1 -10

[5]. (Shambhavi *et al*., 2017). Shambhavi, S., Kumar, R., Sharma, S.P., Verma, G., Sharma, R.P., & Sharma, S.K.(2017) Long-term effect of inorganic fertilizers and amendments on productivity and root dynamics under maize-wheat intensive cropping in an acid Alfisol. *J. Appl. Nat. Sci*. (9), 2004–2012. 1-15

[6]. (dos Santos *et al*., 2020). Dos Santos, R.M., Diaz, P.A.E., Lobo, L.L.B., & Rigobelo, E.C. (2020) Use of Plant Growth- Promoting Rhizobacteria in Maize and Sugarcane: Characteristics and Applications. *Front. Sustain. Food Syst*., 4(*136*), 1 -15

[7] Gajc-Wolska *et al*., 2018 Gajc-Wolska, J., Mazur, K., Niedzi ´nska, M., Kowalczyk, K., & Zołnierczyk, P. (2018) The influence of foliar fertilizers on the quality and yield of sweet pepper (*Capsicum annuum* L.). Folia Hortic. *30*, 183–190.

[8] Seleiman *et al*., 2021). Suleiman, M.F., Almutairi, K.F., Alotaibi, M., Shami, A., Alhammad, B.A., & Battaglia, M.L.(2021) Nano-fertilization as an emerging fertilization technique: Why can modern agriculture benefit from its use? *Plants*, 10(2),1 -15.

[9] (Vidak *et al*., 2021). Vidak, M., Lazarevi´c, B., Petek, M., Gunjaˇca, J., Šatovi´c, Z., Budor, I., & Carovi´c-Stanko, K. (2021) Multispectral Assessment of Sweet Pepper (*Capsicum annum* L.) Fruit Quality Affected by Calcite Nanoparticles. Biomolecules, 11(832), 1 - 13.

[10] Ogunlade, I., Alebiosu, A.A. & Osasona, A.I. (2012)Proximate, mineral composition, antioxidant activity, and total phenolic content of some pepper varieties (Capsicum species). Int. J. Biol. Chem. Sci. 6(5): 2221-2227,

[11] Yanni, Y.G., Dazzo, F.B., Squartini, A. *et al.* Assessment of the natural endophytic association between *Rhizobium* and wheat and its ability to increase wheat production in the Nile delta. *Plant Soil* **407,** 367–383 (2016). https://doi.org/10.1007/s11104-016-2895-0

[12] Rabha M, Sharma S, Acharjee S, Sarmah BK (2017) Isolation and characterization of Bacillus thuringiensis strains native to Assam soil of North East India. 3Biotech 7(5):303 https://doi.org/10.1007/s13205-017-0935-y

[13] Janssen, P.H., Yates, P.S., Grinton, B.E., Taylor, P.M., and Sait, M. (2002), Improved Culturability of Soil Bacteria and Isolation in Pure Culture of Novel Members of the Divisions *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, *Appl Environ Microbiol*,68(5): 2391–2396.

[14] (Di Benedetto *et al*., 2019). Di Benedetto, N.A., Campaniello, D., Bevilacqua, A., Cataldi, M.P., Sinigaglia, M., Flagella, Z., & Corbo, M.R. (2019) Isolation, Screening, and Characterization of Plant-Growth Promoting Bacteria from Durum Wheat Rhizosphere to Improve N and P Nutrient Use Efficiency. Microorganisms (7), 541. 1 -18

[15] (Agbodjato *et al*., 2015). Agbodjato, N.A., Noumavo, P.A., Baba-Moussa, F., Salami, H.A., Sina, H., Sèzan, A., & Bankolé, H (2015). Characterization of Potential Plant Growth Promoting Rhizobacteria Isolated from Maize (*Zea mays* L.) in Central and Northern Benin (West Africa). *Applied and Environmental Soil Science* (2015): 1 - 9

[16] (Lindgreen, 2012) Lindgreen, S. (2012). Adapter Removal: easy cleaning of next-generation sequencing reads BMC Research Notes. 5(337): 1 -7
[17] (Souvorov *et al*., 2018) Souvorov, A., Agarwala, R., & Lipman, D. J. (2018). SKESA: strategic k-mer extension for scrupulous assemblies. *Genome Biol*. 19(*153*),1 -13.
[18] (Pandey & Gupta, 2020). Pandey, S. & Gupta, S. (2020) Evaluation of Pseudomonas sp. for its multifarious plant growth-promoting potential and its ability to alleviate biotic and abiotic stress in tomato (*Solanum Lycopersicum*) plants, *Scientific Reports*, 10 (20951), 1 -15.
[19] Qessaoui, R., Bouharroud, R., Furze, J.N., El Aalaoui, M., Akroud, H., Amarraque, A., VanVaerenbergh, J, Tahzima, R., Mayad, E.H. & B. Chebli, B.(2019). Applications of New Rhizobacteria Pseudomonas Isolates in Agroecology via Fundamental Processes Complementing Plant Growth. Scientific Reports, 9, (12832), 1 – 8
[20] Sokoto, M.B. and Victor, O. (2017). Growth and yield of amaranth (*Amaranthus* spp.) as influenced by seed rate and method of planting in Sokoto, Nigeria. *Archives of Agriculture and Environmental Science*, 2(*1*), 29-35
[21] (dos Santos *et al*, 2020). Dos Santos, R.M., Diaz, P.A.E., Lobo, L.L.B., & Rigobelo, E.C. (2020) Use of Plant Growth- Promoting Rhizobacteria in Maize and Sugarcane: Characteristics and Applications. *Front. Sustain. Food Syst*., 4(*136*), 1 -15
[22] Abbas (2015) Marhoon, I.A., & Abbas, M.K. (2015) Effect of Foliar Application of Seaweed Extract and Amino Acids on Some Vegetative and Anatomical Characters of Two Sweet Pepper (*Capsicum annum* L.) Cultivars. *Int. J. Res. Stud. Agric. Sci*. (1), 35–44