INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
eISSN:2320-3137
Earthjournals Publisher
www.earthjournals.in

RESEARCH ARTICLE

Assessment of gene deletion during antinutrient reduction in *Cucurbita maxima* leaf in Rivers State Nigeria

Tabowei Onuosa Churchill*,, Emylia T. Jaja,Victoria Willson

Rivers State University Department of Plant Science and Biotechnology Port Harcourt.

Corresponding author: Tabowei Onuosa Churchill

Publication history: Received on 6 Sep 2021, Accepted on 8 Oct 2021, Published online 10 Oct 2021

ABSTRACT:

The assessment of gene deletion during antinutritional factors reduction in *Cucurbita maxima (*duchesne linn) winter squash leaf was done using various priming agents: Hydropriming: -biomagnetic water, Bioprime: - biochar and Bacillus licheniformis) and standard control at the Rivers State University, Teaching and research farm Port Harcourt, Seeds and leaf of *Cucurbita maxima* sample plant were collected and analysed using standard methods. The DNA extract from the leaf of Cucurbita maxima were subjected through molecular analysis and the sequenced result inferred that there was deletion in some nucleotide due to the primers used, confirming that some gene was knock down from the mainframe which resulted to reduction in antinutrient and bioavailable of nutritional level of the treated plant. Nonetheless, biomagnetic water had the highest power of efficacy in gene deletion thereby causing highest reduction of nutritional and antinutrient level in *Cucurbita maxima* seed and leaf followed by *Bacillus licheniformis* and Biochar which has the capacity to bioregulate the gene around the plant mechanism due to biomass (*Ipomea aquatica*) use for biochar production.

Keywords: antinutritional, *Cucurbita maxima*, leaf,

INTRODUCTION

WHAT IS PRIMING: Priming could be defined as various treatment processes of seeds in solutions before sowing which could allow the seeds to undergo imbibition to shorten the early phase of germination processes. Thereby disallowing radical appearance from the seed coat (Pirasteh-Anosheh& Hashemi, 2020). Priming could also be viewed as those steps used to bioregulate the processes of early germination of seeds by biocontrolling seed moisture content, cellular cycle bioregulation, equal seed structural and biochemical developmental uniformity, curtail mobilization, suppression of dormancy, management of the environmental condition, encourage stress tolerance, repairs of seed membranes, DNA and RNA repair and synthesis. Seed priming techniques could influence plant growth, nutrient utilization, cost-effectiveness, positive influence on seed germination processes ensures reductions of antinutrient and bioregulate genes responsible in various part of plants(Fiorilli*et al*., 2020).

MOLECULAR INTERACTION BETWEEN PLANTS VIA PLANT DERIVED BIOCHAR, BIOMAGNETIC WATER AND PROBIOTIC BACTERIA: epigenetic

influence on a host plant by microbes, biochar and abiotic factors(biomagnetic water) could be beneficial or antagonistic depending on the DNA and RNAs repairs taking place in the plant molecular structure and availability of nutrient(Fiorilli *et al*., 2020).

PLANT INTERACTION WITH MICROBIAL LIVES: However, Plant symbiotic associations with microbes would be negative or positive depending on the molecular effect generated in the plant mineral nutrient composition*(Capek et al.*, 2018). Plant - microbial interaction could lead to microbial DNA transfer model to the host plant cell repairs also the plant- microbial interdevelopmental phase that would lead to specific organ formation for nutrient generation purposes(Johnson-Green, 2018). Moreover, the expression of the gene responsible for knockdown of phytates and zeatin synthesis in endophytic microbes Bacillus licheniformis was upregulated into host jatropha plant cake and was able to reduce the bioaccumulation of novel antinutrients: phytic acid, trypsin inhibitors, tannins but the lowest among them was lectin antinutrients(Phengnuam & Suntornsuk, 2013). *Albert, Bacillus licheniformis* has been reported to reduce antinutrients and improve the nutritional value of plants also able to control sodium uptake in high saline soil(Zhou *et al.*, 2017).

DNA SEQUENCING: This is any molecular method that could be used to predict the way the four basic forming amino acids(adenine, guanine, cytosine and thymine) in the DNA are arranged it could be the prediction of the ways the four basic nucleotides are found in the DNA. This could also be used to predict the number of guanine and cytosine are present in a DNA molecule (GC rich content of the DNA, RNA, or fragment of the whole genomes)(Singh *et al.*, 2018). However, the knowledge of DNA sequencing could be applied in various discipline ranging from biotechnology, evolutional biology, systematic biology, virology, medical diagnosis, and forensic sciences depending on the research it could be used to determine the riches of hydroxyl methyl or glucose cytosine would replace the cytosine and for bacteriophage in case of virus cytosine would be missing due to deletion leading to DARK DNA or camouflage would result to the deletion of certain genes responsible for the biosynthesis of some micronutrient, macronutrients and antinutrient in plants(Ebbert *et al.*, 2019; Mager & Ludewig, 2018).

DNA SEQUENCING IN PLANT GENOMES: However, the techniques used in sequencing has a great deals in detecting the concentration of GC rich region in a gene and the rate of cytosine DNA methylation that could found in some bacteria, fungi, plant and animal cell (Mager & Ludewig, 2018) . Moreover, the quantity of DNA methylation detected in any plant or bacteria cell would lead to cytosine DNA methylation hence epigenetic and DNA demethylation could lead to different modification of gene involved in nutritional and antinutritional bioaccumulation and biosynthesis(Capuano *et al*., 2014; Xia, 2018). Although, the overexpression of some candidate gene in a host plant through a bacteria vector or Arabinose spp that could express a desired gene by increasing or reducing the concentration or amount of antinutrient in the host plant thereby controlling the effect of abiotic stress or bioregulate the amount of secondary metabolites in host plants hence correlating the amount of gene expressed and the content of antinutrient the gene with higher correlation would upregulated(Huang *et al*., 2020). Moreover, gene resequencing has been used to determine the level of distribution of antinutrient in leaves and seed of plants while the construction of the length of non-codon and codon sequence would be done using semi-

synthetic complementary DNA and a main frame(Liu *et al.,* 2020). However, RBCL gene is a biomarker use during sequencing and PCR forwarding and reversed primers but could perform better when mix with other biomarker(Xue *et al.*, 2019). Thus, the aim of this research has been to determine the gene that are deleted or that determines the reduction of antinutritional factors leading to bioavailability of nutrients in plants.

MATERIALS AND METHODS EXPERIMENTAL LOCATION:

Priming of seeds were carried out in River's state University Biology Laboratory while the field trial was performed at the University Research farm. However, DNA extraction, PCR and GCMS were done in University of Port Harcourt Regional Centre for Bioresearch (RCBBR) then sequencing was carried out at International Institute of Tropical Agriculture (IITA) Ibadan.

SAMPLE COLLECTION AND PREPARATION:

Biopriming Agents:

Biochar was prepared in Rivers State University biology laboratory using Ipomoea aquatica (water spinach-swampy morning glory) in a mini oven at temperature of 350oC for 2 hours and Cucurbita maxima fruit was purchased from Town market Port Harcourt and were identified by Green O. Blessing. Seeds from matured fruits of Cucurbita maxima were extracted from the pulp, washed and air dried for 20 minutes and viable test was done to select seeds that were good for pre-treatment. Bacillus licheniformis pure culture was gotten from international institute tropical agriculture IITA Ibadan, viable cells were revived by subculturing the colonies in a nutrient agar plate, the broth were prepared and used as biopriming agent.

Hydropriming Agents

Biomagnetic treated water were prepared in Rivers University Biology Laboratory by the conversion of the Bio-disc scaler energy to potential energy by the help of rotor machine. However, the pre-treated seeds were covered with Whatman paper, then was transferred to the research farm for field trial. Irrigation was done once a day for four weeks while flowering and fruiting started from 60 days of sowing.

DNA ISOLATION, PCR AND SEQUENCING

Five leaves were harvested in triplicate from each ridge of treatment and was inserted immediately into cooled ice-bag properly labeled while the leaves were transported to University of Port Harcourt Regional Centre for Bioresearch's (RCBBR) for DNA isolation and extraction.

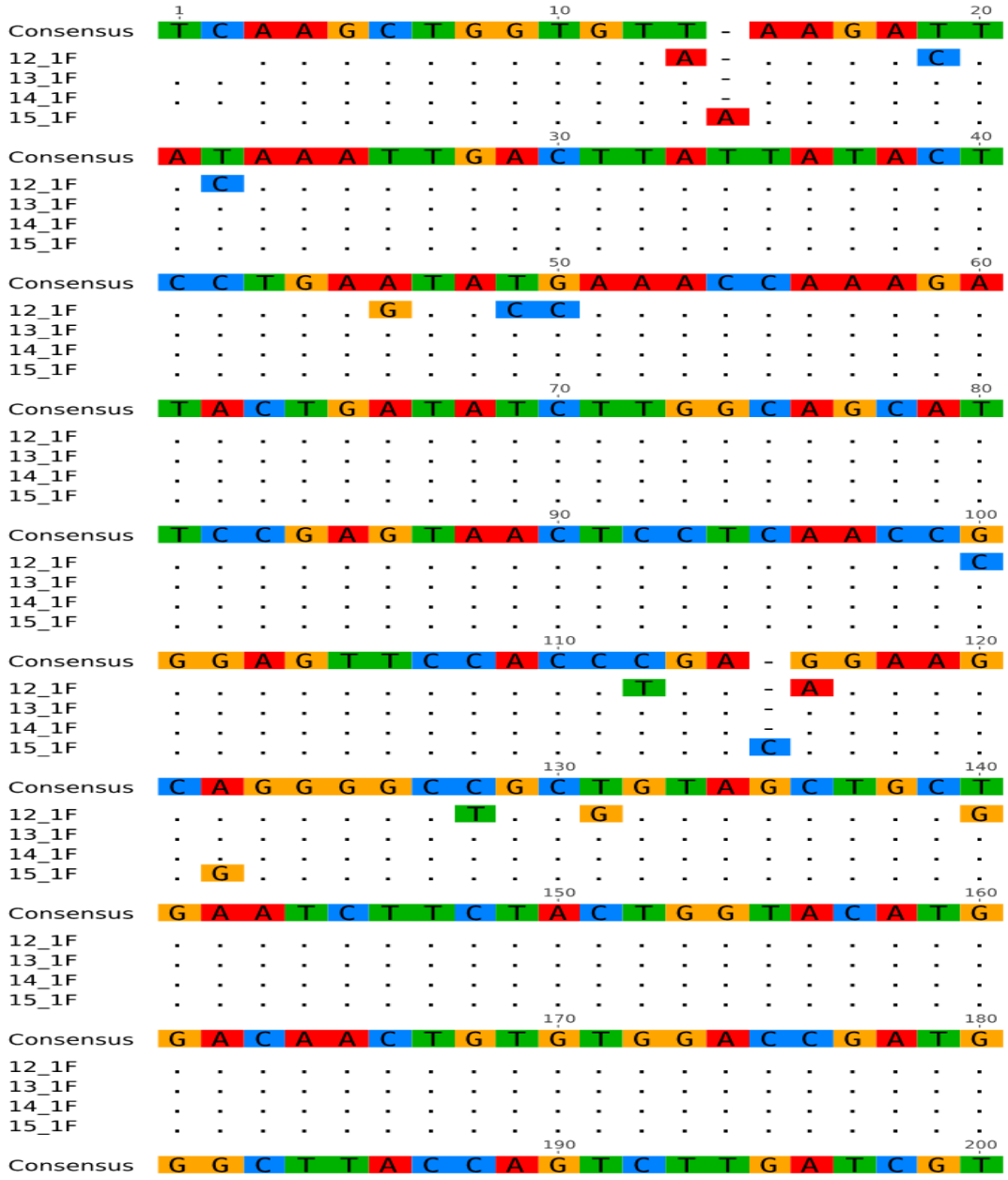
LEAF COLLECTION FOR DNA ISOLATION using ZYMO QUICK DNA PLANT/SEED MINIPREP KIT

Protocol

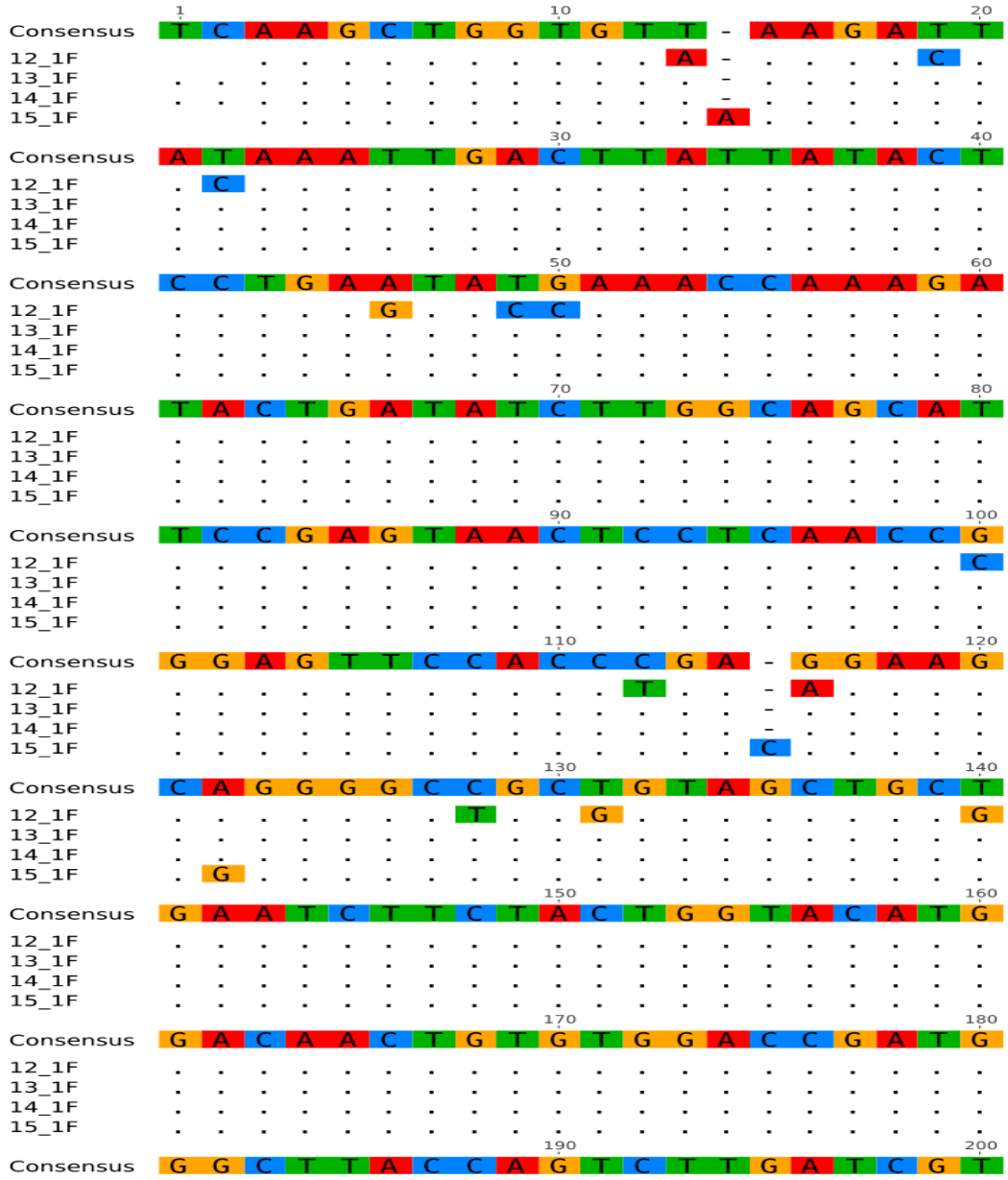
Five leaf each were harvested from each treatment ridge in triplicate and immediately inserted into ice bag and transported quickly to biotechnology molecular research laboratory using a modified method(Ali *et al.*, 2019). Them they were immediately processed in the laboratory following Zymo quick DNA fungal/bacterial miniprep kit protocol were adopted(Illumina, 2014; Sikiru *et al.*, 2017).

INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
Earthjournals Publisher
eISSN:2320-3137
www.earthjournals.in

RESULT



INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
Earthjournals Publisher
eISSN:2320-3137
www.earthjournals.in



Where lane 12_1F represent Biochar treated plant sample amplicon 13_1F represent B. licheniformis, lane 14_1F represent Biomagnetic water treated plant sample and lane 15_1F represent control plant sample amplicons

Figure 1: Effect of priming on the RBCL gene of *Cucurbita maxima* including all the treatment samples

DISCUSSION

The sequenced results showed some variation in the sequenced treatment of biochar still bearing some trait of *Ipomea aquatica* which was a biomass used to produce biochar and that is the only different sequenced having a wide family trait from the control sequence(Yan *et al.,* 2019). However, the Biochar sequencing GC clamp resisted deletion probability due to ability of biochar to introduce the inherent biomass DNA into the host plant nutritional and secondary metabolites composition thereby restructuring the molecular structure of host plant but has more deletion in the C-terminal of the fragment depicting direct and indirect mutation in the RBCL gene and also the limitation of the RBCL gene primers to discriminate plant of different species than plant of genus this is the direction of the author The molecular structure of treated *Cucurbita maxima* with biomass of *Ipomea aquatica* had high phylogenetic alteration probably due to the limitation of the gene of RBCL on species than genus hence linking the biochar sequenced result to the original biomass used for production *Ipomea aquatica* showing its ability to compete favourably in a plant host when applied a biochar in line with (Lakitan & Kartika, 2020)which must have affected the wide mutation that took place in lane _12 of the sequenced sample

CONCLUSION

The antinutritional factors present in*Cucurbita maxima* can be bioregulate by hydropriming agent: Biomagnetic water, and Biopriming agents: Biochar ***and Bacillus licheniformis.*** However, the priming agents has the ability to initiate frameshift mutation thereby resulting to gene deletion confirmed in the sequenced results amongst the priming agents use the deletion took place more in Biomagnetic water primed lane followed by ***Bacillus licheniformis***but the Biochar Lane resisted knockin gene but perform of gene knockdown of host plant genomes thereby superimposing its molecular constitute as observed from the sequencing result. Moreover, the dosage of priming agents use can determine the rate of antinutrient reduced, bioavailable mineral nutrient and inform the deletion rate in the treated plant molecular structure. Although, RBCL gene had its limitation by the inability to give 100% separate in the phylogeny of the genus, when use as biomarker gene for sequencing, also as PCR forward and backward primers.

RECOMMENDATION

More research is needed to know *the* dosage of primers that can be used to achieve a standard transgenic *Cucurbita maxima* with regulated antinutrient.

More molecular study is needed to determine the effect of other primers and GC flanking region of *Cucurbita maxima* that is altered during priming.

REFERENCES

Ali, Q., Salisu, I. B., Raza, A., Shahid, A. A., Rao, A. Q., & Husnain, T. (2019). A modified protocol for rapid DNA isolation from cotton (Gossypium spp.). MethodsX, 6, 259–264. https://doi.org/10.1016/j.mex.2019.01.010

Capek, P., Manzoni, S., Kaštovská, E., Wild, B., Diakova, K., Barta, J., Schnecker, J., Biasi, C., Martikainen, P., Alves, R., Guggenberger, G., Gentsch, N., Hugelius, G., Palmtag, J., Mikutta, R., Shibistova, O., Urich, T., Schleper, C., Richter, A., & Santruckova, H. (2018). A plant–microbe interaction framework explaining nutrient effects on primary production. Nature Ecology & Evolution, 2. https://doi.org/10.1038/s41559-018-0662-8

Capuano, F., Mülleder, M., Kok, R., Blom, H. J., & Ralser, M. (2014). Cytosine DNA methylation is found in Drosophila melanogaster but absent in Saccharomyces cerevisiae, Schizosaccharomyces pombe, and other yeast species. Analytical Chemistry, 86(8), 3697–3702.

Ebbert, M. T. W., Jensen, T. D., Jansen-West, K., Sens, J. P., Reddy, J. S., Ridge, P. G., Kauwe, J. S. K., Belzil, V., Pregent, L., Carrasquillo, M. M., Keene, D., Larson, E., Crane, P., Asmann, Y. W., Ertekin-Taner, N., Younkin, S. G., Ross, O. A., Rademakers, R., Petrucelli, L., & Fryer, J. D. (2019). Systematic analysis of dark and camouflaged genes reveals disease-relevant genes hiding in plain sight. Genome Biology, 20(1), 97. https://doi.org/10.1186/s13059-019-1707-2

Fiorilli, V., Catoni, M., Lanfranco, L., & Zabet, N. R. (2020). Editorial: Interactions of Plants With Bacteria and Fungi: Molecular and Epigenetic Plasticity of the Host. Frontiers in Plant Science, 11, 274. https://doi.org/10.3389/fpls.2020.00274

Huang, Z., Wang, Y., Lu, H., Liu, X., Liu, L., & Xu, A. (2020). Mapping and identification of yellow seed coat color genes in Brassica juncea L. https://doi.org/10.21203/rs.3.rs-25889/v1

Illumina, P. M. (2014). System User Guide. Illumina: San Diego, CA, USA, 1–94.

Johnson-Green, P. (2018). Introduction to food biotechnology. CRC Press.

Lakitan, B., & Kartika, K. (2020). Population density, multiple harvesting, and ability of Ipomoea reptans to compete with native weeds at tropical wetlands. Biodiversitas Journal of Biological Diversity, 21, 4376–4383. https://doi.org/10.13057/biodiv/d210957

Liu, S., Huang, H., Yi, X., Zhang, Y., Yang, Q., Zhang, C., Fan, C., & Zhou, Y. (2020). Dissection of genetic architecture for glucosinolate accumulations in leaves and seeds of Brassica napus by genome-wide association study. Plant Biotechnology Journal, 18(6), 1472–1484. PubMed. (Accessed 10.03.2021)https://doi.org/10.1111/pbi.13314

Mager, S., & Ludewig, U. (2018). Massive Loss of DNA Methylation in Nitrogen-, but Not in Phosphorus-Deficient Zea mays Roots Is Poorly Correlated With Gene Expression Differences. Frontiers in Plant Science, 9, 497–497. PubMed. (Accessed 04.06.2021) https://doi.org/10.3389/fpls.2018.00497

Phengnuam, T., & Suntornsuk, W. (2013). Detoxification and anti-nutrients reduction of Jatropha curcas seed cake by Bacillus fermentation. Journal of Bioscience and Bioengineering, 115(2), 168–172.

Pirasteh-Anosheh, H., & Hashemi, S. E. (2020). Priming, a Promising Practical Approach to Improve Seed Germination and Plant Growth in Saline Conditions. Asian Journal of Agriculture and Food Sciences (ISSN: 2321–1571), 8(01).

Sikiru, G., Yusuf, S., Agbolade, J., & Imonmion, J. (2017). The levels of yield and purity of genomic DNA from five tomato cultivars subjected to two DNA extraction techniques. Nigerian Journal of Biotechnology, 33, 131. (Accessed 20.10.2020) https://doi.org/10.4314/njb.v33i1.19

Singh, A.K. & Mukesh, Kumar & Mitra, Sabyasachi. (2018). Carbon footprint and energy use in jute

and allied fibre production. Indian Journal of Agricultural Sciences. 88. 1305-1316.

Xia, X. (2018). Fundamentals of Proteomics. In Bioinformatics and the Cell (pp. 421-436). Springer, Cham.

Xue, S., Shi, T., Luo, W., Ni, X., Iqbal, S., Ni, Z., Huang, X., Yao, D., Shen, Z., & Gao, Z. (2019). Comparative analysis of the complete chloroplast genome among Prunus mume, P. armeniaca, and P. salicina. Horticulture Research, 6(1), 1–13.

Yan, S., Niu, Z., Yan, H., Zhang, A., & Liu, G. (2019). Transcriptome sequencing reveals the effect of biochar improvement on the development of tobacco plants before and after topping. PLOS ONE, 14(10), e0224556.(Accessed 10.09.2021) https://doi.org/10.1371/journal.pone.0224556

Zhou, C., Zhu, L., Xie, Y., Li, F., Xiao, X., Ma, Z., & Wang, J. (2017). Bacillus licheniformis SA03 Confers Increased Saline–Alkaline Tolerance in Chrysanthemum Plants by Induction of Abscisic Acid Accumulation. Frontiers in Plant Science, 8, 1143. (Accessed 02.08.2020) https://doi.org/10.3389/fpls.2017.01143

Paper cited as: **Tabowei Onuosa Churchill, Emylia T. Jaja,Victoria Willson . Assessment of gene deletion during antinutrient reduction in Cucurbita maxima leaf in Rivers State Nigeria. International Journal of Medical and applied Sciences.2021;10(2): 69-76.**