

ASSESSORS' CONSOLIDATED REPORT ON UPLB'S EE-1 EGGPLANT APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On August 24, 2020, UPLB submitted EE-1 eggplant for direct use, as original application under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016.

After reviewing the Risk Assessment Report and attachments submitted by the applicant, the Scientific and Technical Review Panel (STRP), Bureau of Animal Industry, and BPI Plant Products Safety Services Division concurred that EE-1 eggplant is as safe as its conventional counterpart.

The Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that EE-1 eggplant is safe as its conventional counterpart and shall not pose any significant risk to human health.

The Department of Environment and Natural Resources Biosafety Committee (DENR-BC) considered that EE-1 eggplant poses no significant adverse effect to the environment.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert also recommended for the issuance of biosafety permit for this regulated article after assessing the socio-economic, social and ethical indicators for the adoption of Genetically Modified Organisms.

Background

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors the complete dossier submitted by UPLB. The SEC expert, on the other hand, was provided with special questionnaire on socio-economic, ethical and cultural considerations that have been addressed by UPLB in relation to their application.

INFORMATION ON THE APPLIED EVENTS

EE-1 eggplant was genetically modified using *Agrobacterium tumefaciens*-mediated transformation. EE-1 eggplant contains the transgene *cry1Ac*, derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*, which encodes a Cry1Ac protein that provides protection against the feeding damage by the target insect pest, the eggplant fruit and shoot borer (FSB), *Leucinodes orbonalis*. EE-1 eggplant also contains the transgene *nptII* derived from *Escherichia coli* K-12 strain transposon Tn5 that expresses a neomycin phosphotransferase II enzyme (NPTII), used as a plant selectable marker during the development of EE-1 eggplant.

Countries Where Approvals Have Been Granted

The EE-1 eggplant has been approved for commercial cultivation and for food and feed use in Bangladesh by the National Committee on Biosafety since 2013.

Source: *Public Information Sheet of EE-1 eggplant for direct use*

STRP's Assessment

1. Host Organism

- a. Eggplant is not commonly an allergenic food. There are very few reports of allergic reactions to eggplants. The protein content in eggplants is low and easily denatured on consumption, thus its allergenicity is low. Its uncommon allergenicity is probably from its endogenous histamine content, among other minor components [1][2].
- b. History of safe use is attributed to eggplant fruits as food, and is typically consumed after cooking [1].

2. Donor Organism

- a. Modification introduced in EE-1 eggplant does not change the composition of eggplants and its introduction is not expected to change consumption patterns in the Philippines [1].
- b. The donor organisms are: *Bacillus thuringiensis* subsp. *kurstaki* which has a long history of safe use and *Escherichia coli*, strain K-12 which also has a long history of safe use and is commonly used as protein production systems in many applications [1].
- c. Cry1Ac protein which provides control against eggplant fruit and shoot borer (EF5B) from *B. thuringiensis* subsp. *kurstaki*, is well-known and expressed in many other genetically modified crops, and not known to be toxic or allergenic. NPTII protein which encodes a selectable marker enzyme neomycin phosphotransferase II from *E. coli*, is also not known to be toxic nor allergenic [1].

3. Transformation System

- a. EE-1 eggplant was generated by *Agrobacterium tumefaciens*-mediated transformation with the plasmid vector pMON10518 [1].
- b. The analysis to determine the number of copies of the plasmid DNA and transgenes present in EE-1 eggplant revealed the presence of a single copy of the T-DNA insert and single copies of the Cry1Ac and NPTII expression cassettes [1].
- c. The integrity of the transgenic insert in EE-1 eggplant was demonstrated using Southern blot analyses [1].
- d. The sequence analysis conducted confirmed that there were no truncations, deletions or rearrangements that were identified/determined [1].
- e. Sequencing of the T-DNA insert in EE-1 eggplant identified the presence of approximately 2.6 Kb of vector backbone sequence. This sequence was compared to all known sequences in the NCBI nucleotide database using the BLASTx search tool. Based on this analysis of the identifiable vector backbone in Event EE-1 eggplant, and the intervening nucleotide sequences between these elements, it can be concluded that it is highly unlikely that any functional proteins will be produced in EE-1 eggplant as a result of the presence of these vector backbone sequences [8].

- f. Multigenerational stability of the inserted DNA over six backcross generations and two self-pollinated populations was demonstrated using Southern blot analysis [1].

3. Food and Feed Safety

- a. The enzyme pepsin was used to test the susceptibility of Cry1Ac proteins to proteolytic degradation in standard simulated mammalian gastric fluid (SGF) studies. These SGF studies confirmed that Cry1Ac is rapidly degraded within 30 seconds [1][3][4][5].
- b. Heat inactivation studies with Cry1Ac have been previously conducted which showed the loss of activity of Cry1Ac after heating temperatures above 75°C [1][3][4][5].
- c. Bioinformatics analysis of the Cry1Ac protein expressed in EE-1 event did not show any homology with known toxins [1][3][4][5].
- d. Acute oral gavage of Cry1Ac was performed. No adverse effects were noted after 5000mg/kg were given to test animals [1][3][4][5].
- e. For NPTII, standard simulated gastric fluid containing pepsin was used in the digestibility study. The results showed that NPTII was rapidly digested. No fragments remained after digestion [1][6][7].
- f. NPTII was degraded by heat at temperatures above 95.8°C [1][6].
- g. Bioinformatics analyses were performed to evaluate whether the sequence of the NPTII protein expressed in Event EE-1 eggplants shows any homology with known toxins. The study demonstrated that no homologies were found [1][6].
- h. Acute oral toxicity studies with NPTII were previously conducted at doses up to 5000mg/kg where no adverse effects (NOEL) were detected on test animals [1][6][7].
- i. Genes *cry1ac* and *nptII* are independently expressed and the functional activity are maintained [1].
- j. The promoters used in the genetic modification of EE-1 eggplant P-35S and P-E35S are constitutive promoters and do not target specific organelle [1][8].
- k. No evidence of interactions was observed in any of the studies conducted as part of the comparative assessment. There are no known interactions between Cry1Ac and NPTII as they have very different modes of action [1].
- l. Cry1Ac and NPTII do not interact in any plant metabolic pathway [1].
- m. Four different bioinformatics approaches were used to assess the amino acid sequence similarities of Cry1Ac with any known or putative allergenic proteins. Comparison was made on overall sequence with AllergenOnline database with a sliding window size of 80 amino acids; with the exact matches on any eight contiguous amino acid peptides with the AllergenOnline database, and against all entries in the entire NCBI protein database. These comparisons confirm the lack of potentially significant sequence analysis between Cry1Ac protein and known or putative allergens [1][3].
- n. Bioinformatics analyses were performed to evaluate whether the sequence of the NPTII protein expressed in Event EE-1 eggplants shows any homology with known allergens. The study demonstrated that no homologies were found [1][6].
- o. The highest mean concentration of Cry1Ac protein in any Event EE-eggplant

tissue was estimated at 9.09 µg Cry1Ac/g FW, the Cry1Ac levels represent a maximum of 0.09% of the total protein in the fruit. The estimated protein content of eggplant fruits is 0.98 g protein/100g FW fruit [4].

- p. The highest mean concentration of NPTII measured of 3.26 µg NPTII/g FW represent a maximum of 0.02% of the total protein in the fruit. The estimated protein content of eggplant fruits is 0.98 g protein/100g FW fruit [4].
- q. The results of the bioinformatics analyses confirmed that the insert in Event EE-1 does not lead to the expression of proteins other than Cry1Ac and NPTII and that none of these proteins display any homologies with known allergens. Therefore, following CODEX recommendations, serum screening is not necessary [1][6].
- r. Although some statistically significant differences between Event EE-1-derived UPLB eggplants and their comparators were detected, the relative magnitudes of the differences were considered small, and the differences were within the 99% tolerance interval of all the near-isogenic comparators and reference varieties grown concurrently in the same trials or in the nearby hybrid trial plots, and/or within the range of published literature values. Therefore, these differences are unlikely to be of biological significance or cause adverse effects in humans or animals [1][9].
- s. No significant differences were seen in the comparison of total dietary fiber in Event EE-1 derived UPLB eggplant F₁ hybrid fruits and with their near isogenic comparators. There were statistically significant differences in the levels of total dietary fiber in pooled samples of Open pollinated (OP) varieties M1 and M8 of Event EE-1-derived UPLB eggplant fruits compared to their pooled near-isogenic comparators, but their differences were within 99% tolerance interval [1][9].
- t. There were statistically significant differences in the levels of the minerals but the relative magnitudes of the differences were small and within 99% tolerance interval [1][9].
- u. No statistically significant differences in the mean levels of amino acids were detected in any paired comparisons between Event EE-1-derived UPLB eggplant F₁ hybrids or OP varieties and their near-isogenic comparators [1][9].
- v. All mean amino acid values for the Event EE-1-derived UPLB eggplant F₁ hybrids and OP varieties were within the range of published literature values and within the range of commercial varieties grown concurrently in the same trials [1][9].
- w. For antinutrients, no differences were observed in comparison with commercial varieties except in alpha-flavonoids, but the differences were within 99% tolerance interval [1][9].
- x. All mean values for the Event EE-1-derived UPLB F₁ eggplant hybrids and OP varieties were within the range of published literature values and within the commercial varieties grown concurrently in the same trials [1][9].

STRP's Conclusion

Find scientific evidence that the regulated article applied for direct use is as safe as its conventional counterpart and is not expected to pose greater risk to human and animal

health.

BAI's Assessment

1. Toxicological Assessment

- a. The enzyme used for digestibility of Cry1Ac protein was pepsin. Protein was readily degraded in pepsin enzyme [1][3][4][5].
- b. Functional activity of Cry1Ac protein was reduced to zero percent when subjected at 75°C. Thus, Cry1Ac protein will lose its functional activity but remain an intact protein when subjected to high temperature [1][3][4][5].
- c. The Cry1Ac sequence showed significant alignments with various *Bt* crystal proteins, with E scores as low as 0 and as high as 3e-41. There is no evidence that these proteins are toxic to mammals and birds, only to insects [1][3][4][5].
- d. Acute oral gavage toxicity studies with Cry1Ac and NPTII show that there were no observable treatment-related effects on survival, body weight gain, food consumption or any clinical and pathological changes on the experimental animal [1][3][4][5].
- e. The characterized Cry1Ac protein derived from the common soil bacterium *Bt* was established to be equivalent to the Cry1Ac proteins isolated from EE-1 Eggplant [1][3].
- f. There were no observed large peptide fragments after the digestibility test using SDS-page gel and western blot analysis for both enzymes. These data support the conclusion that NPTII expressed in transgenic plants will be readily digested as conventional dietary protein under typical mammalian gastric conditions [1][6][7].
- g. Functional activity of PAT protein was reduced to 0% when subjected to 95.8°C. Thus, NPTII protein will lose its functional activity but remain an intact protein when subjected to high temperature [1][6].
- h. BLASTP indicated that the NPTII sequence showed no significant amino acid alignments between NPTII and any proteins known or suspected to be mammalian toxins. This indicates that NPTII did not show any homology to known toxins [1][6].
- i. The Cry1Ac and NPTII proteins are expressed independently of each other [1].
- j. The Cry1Ac and NPTII proteins do not interact as they have very different modes of action [1].
- k. The Cry1Ac and NPTII proteins do not interact in a metabolic pathway [1].

2. Allergenicity Assessment

- a. Based on the query search of the given amino acid sequence of Cry1Ac and NPTII in EE-1 eggplant, it was found that there were no similar allergens found in the database (AllergenOnline). There are no sequences found with more than 35% similar identity and no sequences with the same 8-mer match which means it is highly unlikely that the protein could pose any allergenicity/toxicity concerns in animal health [3][6].

- b. The total protein of Cry1Ac in EE-1 eggplant fruit was 0.09% [1].
- c. The total protein of NPTII in any EE-1 eggplant fruit was 0.02%. The mean concentration of NPTII from the fruit of the eggplant ranges from 11.30 to 29.41 ug/g DW (0.95-3.26 ug/g FW) [1].

3. Nutritional Data

- a. Crude protein is significantly higher in the Event EE-1-derived UPLB eggplant Hybrids compared to the control but is within the 99% tolerance interval of all the near-isogenic comparators and within the range of published literature values [1][9].
- b. For the proximate analysis of eggplant fruits (crude fat, crude fiber, crude protein and ash total dietary fiber), significant differences found are within the 99% tolerance interval and do not pose any biological importance to safety [1][9].
- c. Potassium, Iron, and Manganese content of Event EE-1-derived UPLB hybrids and several mineral levels of Event EE-1-derived UPLB OP varieties are significantly different from its non-modified control but is within the 99% tolerance interval of all the near-isogenic comparators. Thus, these differences are not biologically relevant [1][9].
- d. For the levels of amino acids, no statistically significant differences were detected between the Event EE-1-derived UPLB eggplant F₁ Hybrid or OP varieties and their non-modified control [1][9].
- e. For the antinutrients and secondary metabolites, the differences detected do not pose any biological importance to safety and any effect in the nutrient intake or in the normal growth of the poultry or in livestock when it is used as animal feed ingredient is very unlikely [1][9].

BAI's Conclusion

Find scientific evidence that the regulated article applied for animal feed use is as safe as its conventional counterpart and shall not pose greater risk to animal health.

BPI PPSSD's Assessment

1. Toxicological Assessment

- a. Seven *in vitro* assays demonstrated that Cry1Ac protein is rapidly degraded in simulated gastric fluid (SGF) within 30 seconds [1][3][4][5].
- b. The heat activity of *Bt*- produced Cry1Ac protein was determined using functional assay and SDS PAGE. The estimated T₅₀ result for activity of Cry1Ac protein is below 15 minutes when heated at temperatures of 75°C and above [1][3][4][5].
- c. Amino Acid Sequence Comparison with non-redundant protein sequences database using BLASTp showed no significant homology of Cry1Ac to any known toxin [1][3][4][5].
- d. Acute oral gavage toxicity of *Bt*-produced EE-1 Cry1Ac and NPTII proteins administered by gavage to mice at dose of 5000 mg/kg body wt (bw) demonstrated no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology [1][6][7].
- e. The western blot results showed that NPTII protein was digested in pancreatin within 5 minutes and no fragments were detected beyond 5 minutes. The estimated T₅₀ result for pancreatin is below 5 minutes. These results showed that NPTII protein is easily digested in pepsin or pancreatin [1][6][7].
- f. At 95°C and above, the activity of NPTII protein was observed to be at 0% with respect to the control heated at 15 and 30 minutes. The estimated T₅₀ result for activity of NPTII protein is below 15 minutes when heated at temperatures of 95°C and above [7].
- g. Amino Acid Sequence Comparison with non-redundant protein sequences database using BLASTp indicated that the protein sequence of NPTII was homologous to fusion protein (*Feo*) of Hepacivirus C (100% identity), a member of family *Flaviviridae* that is a leading cause of chronic liver disease and cancer. However, the said fusion protein, *Feo*, is not part of the original viral genome. *Feo* was used as a selectable marker for the luciferase assay conducted by Tanabe et al. (2003) to determine the inhibitory effect of ribavirin and interferon- α against Hepatitis C virus (HCV). *Feo* consists of a chimeric gene coding for firefly luciferase protein fused in-frame with *neomycin phosphotransferase* [6][10][11].
- h. Cry1Ac and NPTII proteins are expressed independently of each other as the *cry1ac* and *nptII* genes expression cassettes are separately driven by different promoters in pMON10518 [1].
- i. The Cry1Ac and NPTII proteins do not interact in a metabolic pathway [1].

2. Allergenicity Assessment

- a. Bioinformatics analysis using the full-length sequence, an 80-mer sliding

window and 8-mer exact match in AllergenOnline database did not yield any significant homology of Cry1Ac and NPTII proteins to any known allergen above 35% shared identity [1][3][4].

- b. Based on the highest mean concentration of Cry1Ac (9.09 µg Cry1Ac/g FW eggplant) and the total protein content of eggplants (0.98 g/100g FW), Cry1Ac protein comprises 0.09% of the total protein [1].
- c. Based on the highest mean concentration of NPTII (3.26 µg NPTII/g FW eggplant) and the total protein content of eggplants (0.98 g/100g FW), NPTII protein comprises 0.02% of the total protein [1].

3. Nutritional Data

- a. Based on the statistical analyses, none of the statistical differences between the proximate levels of EE-1 eggplant and non-transgenic eggplant can be considered biologically relevant. All mean values were within the range of commercial varieties. For the total dietary fiber and ash content, the difference of the values from the literature range are attributed to the experiment since both the transgenic and non-transgenic eggplants showed values above the range [1][9].
- b. Based on the statistical analyses, none of the statistical differences between the proximate levels, minerals, amino acids, and anti-nutrients of EE-1 eggplant and non-transgenic eggplant can be considered biologically relevant. All mean values were within the range of commercial varieties. For the total dietary fiber and ash content, the differences of the values from the literature are attributed to the experiment since both the transgenic and non-transgenic eggplants showed values above the range [1][9].

BPI PPSSD's Conclusion

Safety assessment based on the nutritional data indicates that there are no significant differences between the proximate, fiber, amino acid, mineral, anti-nutrient and secondary metabolite levels of EE-1 eggplant and conventional eggplant that can be considered biologically relevant. Weight of evidence approach indicates that EE-1 eggplant is substantially equivalent to its conventional counterpart.

Find scientific evidence that the regulated article applied for direct use is as safe as its conventional counterpart and is not expected to pose greater risk to human health.

DENR-BC's Assessment

1. The regulated article is considered substantially equivalent to its conventional counterpart for its history of safe use for food and cultivation in Bangladesh [19].
2. *Bacillus thuringiensis*, the donor organism for the *cry1Ac* gene is a common soil bacterium that is ubiquitous to the environment. The donor for the *nptII* gene, *Escherichia coli*, is also commonly found in the environment. The *cry1Ac* gene's insecticidal toxin product is specific to the Eggplant Fruit and Shoot Borer (EFSB) and some other lepidopteran pests, with no toxic effects on humans and other organisms [20]. The *nptII* gene only served as a selectable marker which has no function in the grown plant;
3. The regulated article is less likely to harm non-target organisms in case of unintended release. According to impact studies on non-target arthropods, there is no significant difference between arthropod communities found within proximity of non-*Bt* and *Bt* eggplants [21][22]; and
4. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment particularly on biodiversity, as well as the mitigating measures and contingency plan.

DENR-BC's Conclusion

After a comprehensive review and evaluation of the documents and scientific evidence from literature submitted by the UPLB concerning its application for direct use for food, feed, or for processing of EE-1 eggplant, the DENR-BC considered that the regulated article poses no significant adverse effect to the environment.

DOH-BC's Assessment

1. *Bacillus thuringiensis* is a common soil bacterium with a long history of safe use in agriculture. Its genome has provided genes for several proteins toxic to insects.

Escherichia coli is a bacterium found in intestines of people and animals and in the environment. Most *E. coli* strains are harmless and are part of healthy intestinal microflora. Some strains are pathogenic, though these are not used as donors in most transformation cassettes. Strain K-12, the donor strain for *nptII* is not considered pathogenic nor toxin-producing.

2. No significant amino acid alignments were found between Cry1Ac & NPTII and any proteins known or suspected to be mammalian toxins. This indicates that Cry1Ac & NPTII have no meaningful structural similarity to known toxins.
3. Results of the bioinformatic approaches showed the lack of potentially significant sequence similarity between the Cry1Ac & NPTII proteins and known or putative allergens, indicating that these proteins are not known allergen and unlikely to be cross-reactive to known allergens.
4. Statistically significant differences were observed in some analytes, but these were sporadic and small. Moreover, the values observed were within the range of variation observed for these analytes in the near-isogenic comparators and commercial reference varieties grown concurrently. Differences observed were not due to the genetic modification in Event EE-1 derived UPLB eggplants and non-Bt eggplant but could be attributed to effects of genetic background (residual heterozygosity in the OP the lines; hybrids are genetically homogenous) and environmental conditions (effect of FSB infestation on Bt and non-Bt comparator plants, which could have affected the fruit composition).
5. Find that the regulated article applied for Direct Use as Food, Feed or for Processing (FFP) is safe as its conventional counterpart and shall not pose any significant risk to human health.

The following are the observations:

- a. Scientific pieces of evidence from toxicity studies and references, find that the regulated article will not cause significant adverse health effects to human health.
- b. Dietary exposure to the regulated article is unlikely to result in allergic reaction.
- c. The regulated article is as safe as food derived from conventional eggplant varieties.
- d. The regulated article is not materially different in nutritional composition from that of the non-transgenic eggplant or the conventional eggplant.

[13][14][15][16][17][18]

DOH-BC's Conclusion

It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

Based on the evaluation of available literature and dossier documents presented, EE-1 Eggplant is as safe as its conventional counterpart for Direct Use as Food, Feed or for Processing (FFP). Use of this event in its usual context is not expected to pose any new or additional risk to human health.

SEC Expert's Assessment

1. Over 60% of the total production of eggplant in the Philippines is grown in Ilocos, Central Luzon and CALABARZON regions. On average from 2010 to 2019, 37% of the country's eggplant production comes from the Ilocos region [12].
2. The average value of production yearly of eggplant is 4.6 billion pesos. This is about half of a percent of the total value of the top 22 crops grown in the country. Eggplant farmers in small farming communities count on the vegetable as a major source of income [12].
3. The eggplants grown in the Philippines are among the main vegetables consumed by Filipino households.
4. Filipino consumption of eggplants would not be affected by introducing this GM eggplant.
5. Filipino consumers would learn sooner or later that the eggplant they are consuming are safer to eat than before. Pesticide residues are negligible to none at all. This improves in the long run the health of Filipinos.
6. The STRP agreed with the applicant that there will be an upgrading of the eggplant grown in the Philippines from conventional to GM eggplants.
7. Introduction of Bt eggplant will not affect the cultural practices of any ethnic or cultural group.

SEC Expert's Recommendation

The SEC expert recommends for the approval and issuance of the biosafety permit of EE-1 eggplant.

REFERENCES

- [1] Hautea, D. 2020. Supporting Dossier of EE-1 eggplant OECD Unique Identifier MAH-EEØØ1-6 Application for Use in Food, Feed, Import and Processing
- [2] Goodman, R. 2020 Appendix 2: Literature Review of Potential Endogenous Allergens in Eggplant
- [3] Garcia-Alonso, M. 2020. Appendix 10 of the Supporting Dossier EE-1 eggplant OECD Unique Identifier MAH-EEØØ1-6 Application for Use in Food, Feed, Import and Processing
- [4] Agbioworld, 2001. Safety of *Bacillus thuringiensis* proteins used to control insect pests in agricultural crops. Available online: http://agbioworld.org/pdf/bt-short_safet.pdf
- [5] Farias et. al., 2015 Farias, D., Ponte Viana M., Ramos Oliveria G., Olinto Santos V., Moreira Pinto C., Araujo Viana D., Vasconcelos I., Grossi-de-Sa M., and Urano Carvalho A., 2015. Food safety assessment of Cry8Ka5 mutant protein using Cry1Ac as a control Bt protein. *Food and Chemical Toxicology*, 81, 81-91. Available online: <https://doi.org/10.1016/j.fct.2015.04.008> (PDF)
- [6] Vlachos, D. & Associates. 2020. Food and feed safety of neomycin phosphotransferase II (NPTII) produced in EE-1 eggplant OECD Unique Identifier MAH-EEØØ1-6. University of the Philippines Los Baños. College, Laguna. Study Identification DVA2020-NPT2-01. (Source of protein sequence)
- [7] Fuchs, R, Ream J, Hammond B, Naylor M, RM L and Berberich S, 1993. Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Nature Biotechnology*, 1543-1547.
- [8] Gardner, R, Howorth A, Hahn P, Brown-Luedi M, Shepherd R and Messing J, 1981. The complete nucleotide sequence of an infectious clone of cauliflower mosaic virus by M13mp7 shotgun sequencing. *Nucleic Acids Research*, 9, 2871–2898.
- [9] Hautea, D., Masanga, A., & Vlachos, De. 2019. Appendix 9, A Study Report on Comparative Compositional Analyses on Event EE-1 derived UPLB Bt Eggplants and Non-transgenic Conventional Eggplants Grown in Confined Field Trials in the Philippines in 2011-2012
- [10] Sabahi, A., S.L. Uprichard, W.C. Wimley, S. Dash and R.F. Garry. 2014. Unexpected structural features of the hepatitis C virus envelope protein 2 ectodomain. *J. Virol.* 88(18):10280-8.
BLAST. <https://blast.ncbi.nlm.nih.gov/smartblast/smartBlast.cgi>
- [11] Tanabe, Y. Sakamoto, N., Enomoto, N., Kurosaki, M., Ueda, E., Maekawa, S., Yamashiro, T., Nakagawa, M., Chen, C.H., Kamazawa, N., Kakinuma, S. and Watanabe, M. Synergistic inhibition of intracellular Hepatitis C Virus replication by combination of ribavirin and interferon- α . *The Journal of Infectious Diseases* 189:1129-39.
- [12] Philippine Statistical Authority Website, August 11, 2020.
In 2012-13, the Philippine exported a minimal volume (1 metric ton, PSA website) of eggplant. The approval of EE-1 eggplant will help maintain domestic trade of eggplant product and help stabilize prices of vegetable products in the Philippines.
- [13] Environmental Health Risk Assessment Report for Modern Biotechnology and Its Applications – For Direct Use as Food, Feed or Processing (FFP)
- [14] Hautea, D., Rasco, E., & Garcia-Alonso, M. 2020. Appendix 1: Biology and Ecology of Eggplant in the Philippines
- [15] <https://fas.org/biosecurity/education/dualuse-agriculture/2.->

agriculturalbiotechnology/bt-corn.html

- [16] Koch, M. S., Ward, J. M., Levine, S. L., Baum, J. A., Vicini, J. L., & Hammond, B. G. (2015). The food and environmental safety of Bt crops. *Frontiers in plant science*, 6, 283. <https://doi.org/10.3389/fpls.2015.00283>
- [17] <https://www.cdc.gov/foodsafety/communication/ecoli-and-food-safety.html>
- [18] Goodman, R. 2020 Appendix 2: Literature Review of Potential Endogenous Allergens in Eggplant
- [19] ISAAA. 2013. International Service for the Acquisition of Agri-biotech Applications. GM Approval Database. Retrieved September 29, 2020 from <http://www.isaaa.org/grnapprovaldatabase/event/default.asp?EventID=351>
- [20] Jurat-Fuentes JL, & Crickmore N. 2016. Specificity determinants for Cry insecticidal proteins: Insights from their mode of action. *Journal of Invertebrate Pathology*, 142:5-10. DOI: 10.1016/j.jip.2016.07.018
- [21] Navasero MV, Candano RN, Hautea DM, Hautea RA, Shotkoski FA, & Shelton AM. 2016. Assessing Potential Impact of Bt Eggplants on Non-Target Arthropods in the Philippines. *PLOS One*. doi: <https://doi.org/10.1371/journal.pone.0165190>
- [22] Prodhan MZH, Hasan MT, Chowdhury MMI, Alam MS, Rahman ML, Azad AK, I-lossain MJ, Naranjo SE, & Shelton AM. 2018. Bt eggplant (*Solanum melongena* L.) in Bangladesh: Fruit production and control of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee), effects on non-target arthropods and economic returns. *PLOS One*. <https://doi.org/10.1371/journal.pone.0205713>