

Biodiversity Assessment and Environmental Risk Analysis of the Single Line Transgenic Pod Borer Resistant Cowpea

Abraham Isah^{1,2}, Rebecca Wusa Ndana¹, Malann David Yoila¹, Abdulrazak Baba Ibrahim³, Rose Suniso Gidado^{2,4}

¹ Department of Biological Sciences, University of Abuja, Abuja, FCT, Nigeria

² Open Forum on Agricultural Biotechnology (OFAB) in Africa, Nigeria Chapter, National Biotechnology Development Agency, (NABDA), Abuja, FCT, Nigeria

³ Forum for Agricultural Research in Africa, Accra, Ghana

⁴ Department of Agricultural Biotechnology, National Biotechnology Development Agency

Corresponding Author:

Abraham Isah¹

National Biotechnology Development Agency, Umar Musa Yar'dua Way, Lugbe, Abuja, FCT, 900107, Nigeria.

Email address: abraham2637@gmail.com

Abstract

Background: The discussion surrounding biological diversity intensified with the introduction of Nigeria's first transgenic food crop, the Pod Borer Resistant Cowpea. Concerns have arisen regarding whether the transgenic *Maruca vitrata*-resistant cowpea poses a threat to human health and other environmentally beneficial insects. Public apprehension, coupled with social activists' callings for the removal of this crop from the nation's food market, persists. ~~Presently~~However, there is a lack of data to counter the assertion that cultivating Pod Borer Resistant (PBR) cowpea may have adverse effects on biodiversity and the overall ecological system. This research has a multifaceted objective, including an examination of the environmental safety of PBR Cowpea and an assessment of its impact on biodiversity compared to its non-transgenic counterpart, IT97KN.

Methods: Seeds for both the transgenic PBR Cowpea and its isoline were obtained from the Institute for Agricultural Research (IAR) Zaria before planting in various farm sites. Both transgenic and non-transgenic cowpea were cultivated following local cultural practices throughout the experiment. Elaborate taxonomic keys were employed to identify arthropods and other non-targeted organisms. Principal component analysis was used to evaluate potential modifications in all ecological niches of the crops. Diversity indices, including Shannon, Pielou, and Simpson, were analyzed using the lmer function of the R package lme4. The analysis of potential modifications in the dissimilarities of non-targeted organisms' communities was conducted using the Bray-Curtis index.

Results: Examination of ecological species abundance per counting week revealed no disruption in the biological properties of non-targeted species due to the cultivation of transgenic PBR Cowpea. Analysis of species evenness and diversity indices indicated no significant difference between the fields of transgenic PBR cowpea and its isoline. Principal Component Analysis results demonstrated that planting PBR Cowpea did not create an imbalance in the distribution of ecological species. All species and families observed during this study were more abundant in transgenic PBR Cowpea

Formatted: Font: (Default) Calibri

Formatted: Font: (Default) Calibri, Italic

Formatted: Font: (Default) Calibri

fields than in non-transgenic cowpea fields, suggesting that the transformation of cowpea does not negatively impact non-targeted organisms and their communities. Evolution dynamics of the species community between transgenic and non-transgenic cowpea fields showed a similar trend throughout the study period, with no significant divergence induced in the community structure due to PBR Cowpea planting. This study concludes that planting transgenic PBR Cowpea positively influences biodiversity and the environment.

Commented [SKAF1]:

Formatted: Font: (Default) Calibri

Introduction

Biodiversity, a term coined from the word biological diversity, is referred to as the heterogeneity and variability of the total number of biological organisms found within a given habitat or ecosystem at any given time (Roe et al., 2019; Dickson et al., 2019; Meine, 2018; Rawat and Agarwal, 2015). The concept of biodiversity is multidimensional, encompassing genetics, species, and ecology. Several studies, including Tilman et al. (2014) and Malhi et al. (2020), have revealed that the degree of variability of living organisms on earth plays a crucial role in sustaining the ecosystem and could serve as a major indicator for predicting the safety of any environment at any given time. The productivity and efficiency of any agricultural system around the world can be strongly influenced by its varietal and species diversity over an extensive scale of conditions (Pawlak and Kołodziejczak, 2020; Carpenter, 2011; Krishna et al., 2009). Biodiversity also plays crucial roles in contributing to an organism's resiliency to stress and shocks and adaptability to new challenging environmental systems, in addition to being a vital factor in the sustainability system of production and genetic improvement (Vasiliev, 2022; Ortiz et al., 2021). With the deleterious-negative impact of climate change in view, which is already resulting in increased crop pest infestation and decreased agricultural soil fertility globally (Pareek, 2017; Skendpić, et al., 2021; Malhi et al., 2021; Habib-ur-Rahman et al., 2022; Subedi et al., 2023), sustaining and improving the variability of crop and animal genetic resources can no longer be overemphasized as it plays a key role in ensuring the resiliency and stability of living organisms over time.

Commented [SKAF2]: Limit this to three references

Commented [SKAF3]: Very long sentence
Please keep it simple

After about thirty years of the safe use of transgenic crops with more than 3 million hectares planted across Africa (Endale et al., 2022) and their recorded benefits (Gbadegesin et al., 2022; Smyth, 2022), debate and concerns about their environmental effects have continued to persist (Gbadegesin et al., 2022; Gbashi et al., 2021; Smyth et al., 2021; Azadi et al., 2015). Critical among the issues discussed so far is its potential impact on biodiversity (Fernandes et al., 2022; Lucht, 2015). Though a number of studies, including O'Callaghan et al. (2005) and Romeis et al. (2014), have suggested that the insecticidal property of the Cry1Ab protein may be toxic to non-target species, including herbivores, parasitoids, and predators, many of these studies examined the impact of this protein on species in non-natural systems without taking into account ecological interactions or the actual level of exposure of vulnerable stages in natural settings (Dale et al., 2002). Conducting additional studies that take into account complex systems and exposure conditions akin to those encountered in the field could offer more realistic insights into the detrimental effects of Bt crops on non-target organisms (Sears et al., 2001).

Commented [SKAF4]: Limit this to the three

Commented [SKAF5]: You need to provide a short background paragraph about this protein
What is it? It may not seem so obvious for people who are not familiar with the story of transgenic maruca resistant cowpea

85
86 The magnitude and significance of conserving biodiversity have been emphasized in the
87 guidance documents of the European Food Safety Authority (EFSA) (2016) as a major goal in
88 environmental protection. Quantifying biodiversity is a prerequisite for being able to reach
89 set targets. Since Nigeria joined the league of biotech countries after its commercialization
90 of its first transgenic crop, insect-resistant (IR) cotton, in 2018, the general debate in Africa
91 on the potential impact of GM crops on biodiversity has triggered (Endale et al., 2022). The
92 introduction of her first transgenic food crop, pod borer-resistant (PBR) cowpea, in 2019 has
93 further exacerbated these concerns among Nigeria's stakeholders. More apparent among
94 the concerns raised about the safety of the introduction of the transgenic PBR cowpea in
95 Nigeria is its potential to negatively impact species and ecosystem diversity, with key
96 stakeholders speculating that its toxicity to the targeted insect, *Maruca vitrata*, means that
97 there is a strong likelihood that the crop will also be toxic to non-targeted organisms (NTOs),
98 including those that play a vital role in the ecosystem. Currently, there is a paucity of data to
99 refute claims that this transgenic PBR cowpea supports biodiversity and is safe for our
100 environment. This study therefore focuses on the biodiversity assessment of the single-line
101 transgenic pod borer-resistant cowpea, with the aim of evaluating its potential impacts on
102 non-target organisms.

103

104

105 Materials & Methods

106 PBR Cowpea Seeds and its Isoline

107 Seeds of both transgenic PBR Cowpea (SAMPEA 20T) and its Isoline, IT97KN were provided by
108 the Institute for Agricultural Research (IAR) Zaria prior to planting in the various farm site. The
109 Cry1Ab event in the PBR Cowpea was confirmed using the lateral flow strip kits obtained from
110 the Qiagen Inc. at the Mary Halaway Laboratory, Department of Biochemistry, Faculty of Life
111 Sciences, Ahmadu Bello University: 5g, each of transgenic and non-transgenic seeds were
112 mashed separately in two different mortars, after which the extraction buffer was added to
113 each container. The flow strip was then inserted and allowed to stay for about 10 minutes
114 after which the lines were read (Fig S1).

115

116 Experimental Design and Sampling

117 The two cowpea lines, IT97KY and IT97KN were planted in three different farms of the
118 National Biotechnology Development Agency (NABDA) within the period of February – May
119 and August - October 2022 and February - April 2023 using irrigation farming method during
120 the dry season with three replications on each farm site (Fig S2). Both cowpea lines were
121 grown following local cultural practices throughout the experiment. The two crop varieties,
122 transgenic (IT97KT) and its non-transgenic isoline cowpea (ITN7KN) were planted in a
123 randomized block design with 3 replications (Fig S2). The measurement of each plot was
124 estimated at 10m by 15m encompassing eight 30cm interspaced rows with 25cm space
125 between each plant. 3m plain boundaries were created to function as seclusion among plots
126 (Fig. S2). No crop was planted on the three research farms one year prior to the research. In
127 addition, there were no application of any herbicide or insecticide before or during the study
128 period.

Formatted: Font: Italic

Commented [SKAF6]: Very long sentence
Kindly split into two sentences

Formatted: Highlight

Formatted: Highlight

Commented [SKAF7]: The labelling of the cowpea
material used is very confusing
Kindly stick to one labeling system

Formatted: Highlight

Formatted: Highlight

Commented [SKAF8]: IT97KN???

Formatted: Highlight

Formatted: Highlight

Commented [SKAF9]: You mean Replication

Commented [SKAF10]: So what could explain this "All
species and families observed during this study were more
abundant in transgenic PBR Cowpea fields than in non-
transgenic cowpea fields" if there was no application of
insecticide in the field planted with non transgenic cowpea "
what factor could have contributed to the reduction
observed

129

130 Identification of species to family and to functional groups

131 Arthropods and other non-targeted organisms were identified by using suitable and
132 elaborated dichotomous taxonomic keys according to Goulet and Huber (1993), Triplehorn *et al.* (2005) and Jenny *et al.* (2017). The taxonomic grouping was done using the family level as
133 default while in cases where classification based on family level was not obtainable, priority
134 was given to the order and suborder to which the organism belong (Jenny *et al.*, 2017). The
135 individual organisms were further grouped into predator, parasitoid and herbivore ecological
136 functional group. There was no unknown organism recorded all through the study period.
137
138

139 Non-Target Organism Community Structure

140 Possible moderations that may have accrued from planting the transgenic PBR Cowpea was
141 analysed using a precise redundancy analysis (RDA) ordination method called the Principal
142 Component Analysis (PCA) (Vanden-Brink *et al.*, 2009) as recommended by Cuppen *et al.*
143 (2000) and Moser *et al.* (2007) to be suitable for assessing the impacts of any plants or animals
144 on the ecosystem. The PCA multivariate technique facilitates the understanding of the
145 interaction between the organism and their environments (Moser *et al.*, 2007) by analysing
146 the possible effects of the transgenic PBR Cowpea on the community species and the resulting
147 changes in the community structure throughout the study period.
148

149 Structural Dissimilarity analysis

150 The analysis for the potential modification in the dissimilarities of the non-targeted
151 organisms' communities between the transgenic PBR cowpea (IT97KT) and its non-transgenic
152 isoline (IT97KN) were done using Bray-Curtis index. It evaluates the degree of dissimilarity or
153 similarity between two or more samples using a range of zero (similar) to one (dissimilar)
154 (Krebs, 1989; Bray and Curtis, 1957). The structural dissimilarity analysis was divided into two
155 phases. At the first phase of the analysis, the Bray Curtis (BC) Index was ~~computed~~
156 using the data collected between all the pairs of the sample plots IT97KT and IT97KN on each
157 sampling date. Similar procedures were repeated for the second phase of the analysis where
158 data was collected within each cowpea plot (Collins *et al.*, 2000) and was then followed by a
159 computation of the mean abundance for the respective taxonomic group in line IT97KT and
160 IT97KN per sampling date.

161 The Bray-Curtis Dissimilarity was calculated as: $BC_{ij} = 1 - (2 * C_{ij}) / (S_i + S_j)$

162 Where C_{ij} = The sum of the lesser values for the species found in each site.

163 S_i : The total number of specimens counted at site i

164 S_j : The total number of specimens counted at site j

165 The values for the mean abundance were thereafter used to estimate the BC distance
166 between the respective treatment sampling dates. A linear regression analysis of the data
167 obtained from the BC distance estimation was conducted versus the time-lag data.
168

169 Statistical Analysis

170 The total number (N) of arthropods on each plot in the three different farm sites were taken
171 per counting week and over the entire period of the study and then divided by the total
172 number of farm site to get the average. All statistical analyses were performed by using R
173 version 4.2.0 (R Core Team, 2022) and excel spread sheet. The analysis of the diversity indices
174 including Shannon (H), Pielou (J) and Simpson (D) which facilitates a comparative assessment
175 of the community structures between different treatments in the fields (Boyle *et al.*, 1990;

176 Magurran, 2004; Pielou, 1966; Oksanen, 2013) were done using the lmer function of R
177 package lme4 with Cowpea variety (Bt or non-Bt) and time (date of sampling) as fixed factors
178 (Guo *et al.*, 2014). A comparison of the mean values of all the scoring parameter including H,
179 D, J and N was done using one-way analysis of variance (ANOVA). Population less than 1%
180 were denoted as “others”.

Commented [SKAF11]: this is not

182 A covariance analysis was used to carry out a comparative study of the slopes of the
183 regression lines of the two treatments. The parasitoid, herbivores and predator nutritional
184 relationships were used to classify the whole organisms into three guilds according to Heong
185 *et al.* (1991) and Zhang *et al.* (2011). The density of the three guilds were analysed using One-
186 way ANOVA for each variety of the cowpea and sampling date. The population of various
187 treatments, herbivore, parasitoid and predator nutritional guild was defined by sing the
188 formula $P_i N_i / N$, where the population of the herbivore, parasitoid and predator was
189 connoted as N_i while the treatment's entire summed abundance was connoted as N. The
190 specie count for each community organism in the various guild was defined by the formula
191 $P_i N_i / N$, where N_i was defined as the summed ith species and N was the guild count in the
192 respective treatment. The rare, common and dominant group were denoted by $P_i < 1\%$,
193 $1\% \leq P_i < 10\%$ and $P_i \geq 10\%$ respectively (Li and Liu, 2013).

Formatted: Normal, Justified

200 **Results**

201 **Transgene Status Confirmation of the Cowpea Samples**

202 The confirmation of the Cry1Ab event expressed in the PBR Cowpea shows a positive result
203 as seen in **Figure S1**. Further test for the presence of the Cry1Ab gene using the event
204 specific flow strip in the isoline of the PBR Cowpea shows negative, meaning that the isoline
205 is not transgenic **Figure S1**.

Commented [SKAF12]: Figure s1 is confusing
I wish you provide a more clear picture with the labels of
each event you are referring to

207 **Ecological Pattern of the transgenic and Non-Transgenic Cowpea Field**

208 Study of the species disparities and distribution shows that there are no significant
209 variations between both treatments during the first three weeks after planting in all the
210 experimental site as seen in **Figure 1, Table 1**. At week 4, 5, 6 and 7, significant difference
211 was observed between both treatments with the field of transgenic crop having higher
212 species activities than the field of non-transgenic cowpea.

Commented [SKAF13]: I am now very confused which
threshold are using to declare whether the differences
between treatments are significant 5%, 1% or 0.1%

Commented [SKAF14]: If I refer to the species count
from week 2 to week 11 there seems to be a difference
between the treatments at 5%
Again what is your probability threshold ????

214 **Estimated Species Diversity**

215 From the results of the univariate analyses of both line IT97KT and IT97KN ecological niches,
216 the estimated biodiversity indices (H, J and D) revealed that there was no significant
217 difference between the two treatments except during the differentiated flowering time that
218 was observed between the two cowpea lines (**Table 1 and Figure 2 a, b and c**). The habitat
219 information provided from the Shannon diversity index analysis shows that both habitats

Commented [SKAF15]: So now you are considering 5%
level of significance
If so you need to revisit some of the above stated
interpretations

Commented [SKAF16]: Kindly crosscheck that graph

dominated by the transgenic and non-transgenic cowpea has high species richness and evenness throughout the counting weeks. Results obtained from the analysis using the Shannon diversity index revealed a close-range value between the transgenic and non-transgenic cowpea habitats. A higher Shannon score was observed for transgenic cowpea fields within the counting week of 3 to 8 where flowering was peak. The diversity index score for IT97KN went slightly high at the counting week where its flowering was also peak. Result from the analysis of variance shows no significant difference at week 1, 2, 9, 10, 11 and 12 against the subsequent counting week of 4, 5, 6 and 7 (Figure 2a). Analysis of the Simpson diversity indices shows similar trends in both fields of the transgenic and non-transgenic cowpea. With both fields recording their lowest Simpson score at week 1 and 2 respectively, the highest Simpson score for transgenic and non-transgenic cowpea fields were recorded at week 12 and 11 respectively (Figure 2b). Analysis of the Pielou Evenness Index shows that the distribution of the individual species is even across the habitat of transgenic and non-transgenic cowpea (Figure 2c).

Further analysis using the regression line plot between the ecological niches of transgenic and non-transgenic cowpea shows strong positive correlation with a p and r value of 1.810599e-06 and 0.9522146 respectively (Figure 3a). As the number of species in the ecological niches of PBR Cowpea increases, the number of species in its non-transgenic isolate, IT97KN also increases (Figure 3a).

Similar results were observed when the ecological niches of transgenic Cowpea (IT97KT) and its non-transgenic isolate (IT97KN) were correlated with time (Figure 3b). The p and r values of 3.42862e-09 and 0.9865187 respectively were observed for transgenic cowpea vs time while p and r values of 1.535e-07 and 0.9522146 respectively were observed for non-transgenic cowpea vs time (Table 2).

Analysis of the Evolution Dynamics of the Transgenic and Non-Transgenic Cowpea

Component Analysis

Analysis using the multivariate Principal Component Technique reveals that there are no significant differences in the ecological composition of the entire study fields throughout the counting weeks (Figure 4a and b). The essence of the Principal Component Analysis (PCA) output is to give a clear interpretation of the species points that have similar composition. The species scores which are represented by arrows point in the direction of increasing abundance. The angle size between a species arrow to another species arrow is inversely correlated, meaning that the smaller the angle size between two species arrows, the stronger the correlation and the reverse means a weaker correlation within the space. As observed from the result output, there is strong positive correlation between EI and DC in both field of transgenic and non-transgenic cowpea field. The formation of a right angle between two species arrows means that there is no correlation while the formation of opposite angle means a strong negative correlation (BioTuring, 2018; Hartmann et al., 2018). The PCA output generated below also attributes significant value to the direction of

Commented [SKAF17]: But not always statistically significant so this cannot be generalized

Commented [SKAF18]: You need to adopt one abbreviation for the lines and stick to that throughout the text
Bt NBt or ITK97T ITK97N not both

Formatted: Font: Bold

Commented [SKAF19]: You need to add a complete legend to figure 4 a and b

Commented [SKAF20]: Are you expecting the readers to guess these
Kindly define for the first time use

the species arrow with respect to its angle with the principal component axes within the space. The more parallel a species arrow is to the axis of a principal component, the more weight they have on that principal component space (Hartmann et al., 2018). The PC analysis from this study shows that AC and Cs strongly influences PC1 while PP and Zv strongly influences PC2, having a heavier weight in the transgenic cowpea field. Md and SaC are the most heavily weighted in PC1, strongly influencing the PC1 of the non- while GB and PP are the most heavily weighted species of PC2 in the non-transgenic cowpea field. The estimation of the number of statistically significant principal components for the ecological niches of both transgenic and non-transgenic cowpea is presented in **Figure 5a and 5b** below. The number of break point (10) distribution is similar for both ecological niches.

272

273 **Composition of Organism Community of both the Transgenic and Non-Transgenic Species**

274 As shown in the figure below, three major guilds, herbivores, parasitoids and predators
275 were identified throughout the study period (**Figure 6a, b and c**). The guild analysis for both
276 the Bt (IT97KT) and NBt (IT97KN) field reveals the identification of 12, 8 and 7 different
277 species in the herbivore, parasitoid, and predator guild. Most of the species in both fields
278 are herbivores while the predatory guild has the least number of organisms. SC represents
279 the most abundant species in the parasitoid guild of both IT97KT and IT97KN ecological
280 niches, while MB and AC are the most abundant species in the herbivore guild. CaC is the
281 most abundant species in the predator guild. SL, LM and vf represent the least abundant
282 species in the predator, parasitoid and herbivore guild of both ecological niches as shown in
283 the figure. A uniform composition of the organisms in all the ecological niches were
284 observed throughout the whole study period (**Figure 6a, b and c**).

285

286 **Dissimilarity Index**

287 The result of the Bray Curtis dissimilarity Index is presented in **Table 3** below. Bray-Curtis
288 Dissimilarity ranges between 0 and 1, where 0 indicates that the niches have no dissimilarity
289 while 1 indicates that the two niches have complete dissimilarity. The dissimilarity index
290 between the ecological niches of PBR Cowpea and non-transgenic isoline is 0.2, which
291 indicates that all the niches had similar evolutionary trends with no divergence in the
292 community structure of the NTOS.

293

294 **Discussion**

295 In this study, the potential impact of Nigeria's transgenic Pod Borer Resistant (PBR) Cowpea,
296 which is the first transgenic cowpea to be commercialised in the world, was assessed with
297 the aim of evaluating the possible threats and harm that the crop may pose to the
298 environment and the ecological niches of the diverse useful soil and plant organisms. ~~Bray~~
299 ~~Curtis-Dissimilarity Index, Pielou Evenness Index, Shannon Diversity Index and Simpson~~
300 ~~Diversity Index have been strongly recommended by Guo et al. (2014) and Clergue et al.~~
301 ~~(2005) as useful measures and indicators to gaining insight and understanding of the impact~~

Commented [SKAF21]: Clarifying this in the methodology section will help avoid citing authors in your results section

Commented [SKAF22]: What are these ???

Commented [SKAF23]: Which one
You better indicate the figure number

Commented [SKAF24]: You need to indicate the meaning of these abbreviations MB AE EL vf DA SS AC DC JO BT CB CaM
All the figures should stand alone

Commented [SKAF25]: Kindly move this to the methodology section

Commented [SKAF26]: ????

of any plant on the community structure, evolution dynamics and ecological pattern of other species and the environments where they are found.

According to Guo et al. (2014), the various functional ecological indices of the surrounding species to any newly introduced crop such as the PBR Cowpea would be significantly altered if disruption of any biological property occurs as a result of planting such crop. However, the findings from this research shows that the total species count throughout the study period are similar in values. Analysis of the various ecological indices, including Shannon Diversity index, Brays Curtis Dissimilarity Index, Pielou evenness index, Principal Component Analysis (PCA) and Renyi Diversity Silhouettes all showed a close range of values between the ecological niches of the transgenic Cowpea and its non-transgenic Isoline. Similar research study conducted at the Germany's Oderbruch European Corn Borer infestation area by Schorling and Freier (2006) on a Six-year assessment of the impact of transgenic maize expressing Cry1Ab gene on non-target organisms reported the same results. In contrast to Fernandes et al. (2022) who postulated that genetic modification of crop has the tendency of reducing crop biodiversity, research findings by Abdul et al. (2022) and Anderson (2019) indicated has underscored that the transformation of crops for insect resistance is beneficial because it can enable plant species that are near extinction due to the heavy burden of insect infestation to be revived by improving their adaptation to diverse environmental conditions.

The Principal Component Analysis (PCA) of both the transgenic and non-transgenic Cowpea fields reveals that the distribution of the NTOs were not significantly different throughout the study period. This finding is consistent with the report of Guo et al. (2014) and Candolfi et al. (2004) who reported where they found out that the Cry1Ab event expressed in the transgenic Corn does not affect the community structure of the NTOs. Other research study by Higgins et al. (2009) where a three-year field monitoring of the potential impacts of Cry1F events expressed in a maize hybrid on NTOs also showed underscored that the community structure of the organisms remained intact. Though previous research study only centred on the comparative NTO abundance between transgenic and non-transgenic plots, the present is study further analysed the possible evolutionary dynamics of the transgenic PBR Cowpea by carrying out a dissimilarity index analysis. The results of this study show s that there was a gradual change in the species composition of both transgenic field and non-transgenic field and this change increased with time. For instance, the species type present during the counting week 2 of the study increased when compared to week 1. Similar occurrence was also observed when counting week 3 was compared with counting week 2. Analysis of the Bray Curtis Dissimilarity Index showed an index of 0.2 , suggesting which means that the evolutionary dynamic for both transgenic and non-transgenic crops were significantly similar. Similar studies conducted by Guo et al. (2014) also recorded a similar evolutionary dynamic between non-transgenic and transgenic maize expressing Cry1Ac event.

The potential toxicity of PBR Cowpea can also be carried out by monitoring and evaluating the exposure of the different life stages of the various species of Cowpea ecosystem (Devos et al., 2012). The assessment of the different nutritional guild of organisms identified in this

Commented [SKAF27]: Implying what in relation to your findings

Kindly can you discuss your results

Commented [SKAF28]: Okay
It was already stated in the results section

study shows a rich representation of the herbivores, parasitoids and predators in all the ecological niches. Despite the high tendency of herbivores having a direct exposure to the Cry proteins expressed in the PBR Cowpea when feeding on its crop residue and pollen (Devos et al., 2012; Romeis et al., 2008), a high population density was still recorded for the PBR cowpea ecological niches when compared to the non-transgenic Cowpea. The number of herbivore species present in the ecological niches of transgenic cowpea is higher than in the non-transgenic cowpea ecological niches but the same species type including: *Messor barbarous*, *Alydus eurinus*, *Eastern Lubber*, *Variegated fritillary*, *Deudorix antalus*, *Scarabaeus satyrus*, *Atta cephalotes*, *Dysdercus cingulatus*, *Junonia oenone*, *Chorthippus biguttulus* and *Carausius morosus* were observed for all the ecological niches. This result is in line alignment with findings from Wolfenbarger et al. (2008) who carried out a study on the potential impacts of GM Crops on the functional guild of NTOs.

A further critical analysis of the population density of the predator guild in both transgenic and non-transgenic field revealed no significant difference. Assessing the population density of the predator guild can provide valid assertions on the extent of biological, as well as environmental safety of the transgenic crop since predators have multiple ways by which they come in contact with the Cry1Ab gene including direct feeding on the pollen of the PBR Cowpea, herbivores that have feed on PBR Cowpea or via the surrounding soil in which the PBR Cowpea is planted. The number of predator species present in the ecological niches of transgenic cowpea is higher than in the non-transgenic cowpea ecological niches though both had the same species type including *Chilocorus stigma*, *Odontoponera transversa*, *Conozoa hyaline*, *Camponotus cruentatus*, *Pirata piraticus*, *Graphoderus bilineatus* and *Stenolophus lecontei*.

Analysis of the parasitoid population can provide some very useful ecological indices because they possess the unique characteristics of having the ability to complete their life cycle by feeding on a particular host (Salama and Zaki, 1983) or a range of herbivores in a particular ecological niche (Romeis et al., 2008). They are therefore most likely to ingest the Cry protein in the host herbivore where they are found or directly from the PBR Cowpea plant (Lit et al., 2012). The analysis shows that the population density of the parasitoids in the PBR Cowpea ecological niches were not significantly different from the non-transgenic Cowpea ecological niches throughout the study period. Studies by Comas et al. (2014) and Albajes et al. (2013) who carried out meta-analysis of the ecological impact of Bt Maize on NTOs also reported that the transgenic maize had no significant effect on the population density of the predator, herbivore and parasitoid guild throughout the study period.

The result of the Principal Component Analysis (PCA) shows similar evolutionary dynamics in both the ecological niches of the transgenic and non-transgenic Cowpea. The broken stick distribution which models the number of variances by adopting a stick of unit length which is thereafter randomly broken into n pieces reveals no statistically significant difference between both ecological niches. This finding is in line alignment with result obtained by Guo et al. (2014) whose research study revealed that the BtCry1Ac event expressed in the insect

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Commented [SKAF29]: Kindly reformulate this

resistant corn caused no alteration in the community distribution of both transgenic and non-transgenic corn.

The strong positive correlation between both transgenic and non-transgenic cowpea vs time shows that the increase in the species in both niches is as a result of ~~increase in agronomic factors as the growth of both cowpea progresses. Such factors may include the onset of flowers and the steady increase, the onset of pods which followed thereafter and its steady increase, in addition to the continuous increase in the number of leaves over time. It also means that the Cry1Ab gene expressed in the PBR Cowpea had no negative impact on any of the ecological components including the non-targeted organisms. Other factors that may have played significant roles include temperature, rainfall, sunshine, nature of the soil and other surrounding elements and plants (Desneux and Bernal, 2010).~~

Limitation of the Current Study

~~The current study does not take the effect of the PBR Cowpea on egg laying capacity of non-targeted arthropods in contrast to Dang et al. (2017).~~ Furthermore, the collection of data on the effect of PBR Cowpea on soil invertebrates over longer periods of time and the potential transfer of the Cry1Ab gene to conventional cowpea still need to be assessed.

Conclusions

The findings from this study shows that the introduction of the *Cry1Ab* transgene in the PBR cowpea did not negatively impact biodiversity and the environment. The comparative assessment of the evolutionary dynamics of the non-targeted species community of the transgenic cowpea and that of non-transgenic cowpea recorded no significant divergence throughout the study period. The data accrued from the analysis of the species evenness and diversity indices also did not show any significant difference between the fields of transgenic PBR cowpea and its isolate. ~~The findings~~ ~~The data accrued~~ from this research ~~are useful in~~ provide ~~ing~~ valuable insights that will help to shape decision-making for the regulation of the crop across ~~the all cowpea major growing areas in the country. -countries where it can be grown.~~

References

- Abdul, A.M., Brini, F., Rouached, H. and Masmoudi, K. (2022). Genetically engineered crops for sustainably enhanced food production systems. *Front. Plant Sci.* 13:1027828. doi: 10.3389/fpls.2022.1027828.
- Albajes, R., Lumbierres, B., Madeira, F., Comas, C. and Ardanuy, A. (2013). Field trials for assessing risks of GM maize on non-target arthropods in Europe: the Spanish experience. *IOBC/WPRS Bulletin* 97: 1–8.
- Anderson, J., Ellsworth, P., Faria, J., Head, G., Owen, M. and Pilcher, C. (2019). Genetically engineered crops: Importance of diversified integrated pest management for agricultural sustainability. *Front. Bioeng Biotechnol.* 7, 24. doi: 10.3389/fbioe.2019.00024
- Azadi, H., Samiee, A., Mahmoudi, H., Jouzi, Z., Khachak, P.R., DeMaeyer, P. and Witlox, F. (2015). Genetically modified crops and small-scale farmers: main opportunities and

challenges. *Critical Reviews in Biotechnology* 36(3):434-446. doi:10.3109/07388551.2014.990413.

Boyle, T.P., Smillie, G.M., Anderson, J.C. and Beeson, D.R. (1990). A sensitivity analysis of nine diversity and seven similarity indices. *Res J Water Pollut Control Fed* 62: 749–762.

Bray, J.R. and Curtis, J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 27: 325–349.

Carpenter, J.E. (2011). Impact of GM crops on biodiversity. *GM Crops*. 2(1):7-23. doi: 10.4161/gmcr.2.1.15086.

Clergue, B., Amiaud, B., Pervanchon, F., Lasserre-Joulin, F. and Plantureux, S. (2005). Biodiversity: function and assessment in agricultural areas. A review. *Agron Sustain Dev* 25: 1–15.

Collins, S.L., Micheli, F. and Hartt, L. (2000). A method to determine rates and patterns of variability in ecological communities. *Oikos* 91: 285–293.

Comas, C., Lumbierres, B., Pons, X. and Albajes, R. (2014). No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. *Transgenic Res* 23: 135–143.

Cuppen, J.G.M., Van den Brink, P.J., Camps, E., Uil, K.F. and Brock, T.C.M. (2000). Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. *Aquat Toxicol* 48: 233–250.

Dale, P.H., Clarke, B. and Fontes, E.M.G. (2002). Potential for the environmental impact of transgenic crops. *Nat. Biotechnol.* 20, 567–574.

Dang, C., Zhou, X., Sun, C., Wang, F., Peng, Y. and Ye, G. (2021). Impacts of Bt rice on non-target organisms assessed by the hazard quotient (HQ), *Ecotoxicology and Environmental Safety*, Volume 207, 111214, ISSN 0147-6513, <https://doi.org/10.1016/j.ecoenv.2020.111214>.

Desneux, N. and Bernal, J.S. (2010). Genetically modified crops deserve greater ecotoxicological scrutiny. *Ecotoxicology* 19: 1642–1644.

Devos Y, De Schrijver A, De Clercq P, Kiss J, Romeis J (2012) Bt-maize event MON 88017 expressing Cry3Bb1 does not cause harm to non-target organisms. *Transgenic Res* 21: 1191–1214.

Dickson, A., Krishnan, U., Parisa, Z., Barbara, S. and Paul, S. (2019). The Concept of Biodiversity and its Relevance to Mankind: A Short Review. *Journal of Agriculture and Sustainability*, Volume 12, Number 2, 219-231

EFSA. (2016). Guidance to define protection goals for environmental risk assessment in relation to biodiversity and ecosystem services. *EFSA Journal*, Volume 14, issue 6: doi: <https://doi.org/10.2903/j.efsa.2016.4499>.

Endale, G.K., Karim, M., Joseph, G. and Muffy, K. (2022). Commercialization of genetically modified crops in Africa: Opportunities and challenges. *African Journal of Biotechnology*; Vol. 21(5), pp.188-197, DOI: 10.5897/AJB2021.17434

Fernandes, G., Silva, A., Maronhas, M., Dos Santos, A. and Lima, P. (2022). Transgene flow: Challenges to the on-farm conservation of maize landraces in the Brazilian semi-arid region. *Plants (Basel)*. 11 (5), 603. doi: 10.3390/plants11050603

Gbadegesin, L.A., Ayeni, E.A., Tettey, C.K., Uyanga, V.A., Aluko, O.O., Ahiakpa, J.K., Okoye, C.O., Mbadiana, J.I., Adekoya, M.A., Aminu, R.O., Oyawole, F.P. and Odufuwa, P. (2022). GMOs in Africa: Status, adoption and public acceptance, *Food Control*, Volume 141, 109193, <https://doi.org/10.1016/j.foodcont.2022.109193>.

474 Gbashi, S., Adebo, O., Adebiyi, J.A., Targuma, S., Tebele, S., Areo, O.M., Olopade, B., Odukoya,
 475 J.O. and Njobeh, P. (2021). Food safety, food security and genetically modified
 476 organisms in Africa: a current perspective. *Biotechnology and Genetic Engineering*
 477 *Reviews*, 37(1):30-63. doi: 10.1080/02648725.2021.1940735.
 478 Goulet, H. and Huber, J.T. (1993). Hymenoptera of the World: An Identification Guide to
 479 Families. *Agriculture*, Canada, Ottawa.
 480 Guo, Y., Feng, Y., Ge, Y., Tetreau, G. and Chen, X. (2014). The Cultivation of *Bt* Corn Producing
 481 *Cry1Ac* Toxins Does Not Adversely Affect Non-Target Arthropods. *PLoS ONE* 9(12):
 482 e114228. doi:10.1371/journal.pone.0114228.
 483 Habib-ur-Rahman M, Ahmad A, Raza A, Hasnain MU, Alharby HF, Alzahrani YM, Bamagoos
 484 AA, Hakeem KR, Ahmad S, Nasim W, Ali S, Mansour F and EL Sabagh A (2022) Impact
 485 of climate change on agricultural production; Issues, challenges, and opportunities in
 486 Asia. *Front. Plant Sci.* 13:925548. doi: 10.3389/fpls.2022.925548
 487 Heong, K.L., Aquino, G.B. and Barrion, A.T. (1991). Arthropod community structures of rice
 488 ecosystems in the Philippines. *B Entomol Res* 81: 407–416.
 489 Jenny, L., Marcel, D., Cajo, J.F.T. and Joop, J.A.V. (2017). Biodiversity analyses for risk
 490 assessment of genetically modified potato. *Agriculture, Ecosystems and Environment*.
 491 Volume 249, 1,196-205
 492 Krebs, C.J. (1989). Ecological methodology. *Harper and Row*, New York.
 493 Krishna, V., Zilberman, D. and Qaim, M. (2009). Transgenic technology adoption and on-farm
 494 varietal diversity. *International Association of Agricultural Economists Conference*.
 495 Beijing, China 2009.
 496 Li, X.G. and Liu, B.A. (2013). A 2-year field study shows little evidence that the long-term
 497 planting of transgenic insect-resistant cotton affects the community structure of soil
 498 nematodes. *PLoS ONE* 8: e61670.
 499 Lit, I.L., Caasi-Lit, M.T., Benigno, E.A., Ramal, A.F.B. and Yap, S.A. (2012). Non-target organisms
 500 on *Bt* corn hybrids MON89034 and MON89034/NK603: Part 2. Functional guilds of
 501 arthropods in regulated field trial sites during dry season in Luzon and Mindanao,
 502 Philippines. *Philipp Entomol* 26: 28–53.
 503 Lucht, J.M. (2015). Public Acceptance of Plant Biotechnology and GM Crops. *Viruses*.
 504 30;7(8):4254-81. doi: 10.3390/v7082819.
 505 Magurran, A.E. (2004). Measuring biological diversity. *Blackwell Science*, Oxford.
 506 Malhi, G.S.; Kaur, M.; Kaushik, P. (2021). Impact of Climate Change on Agriculture and Its
 507 Mitigation Strategies: A Review. *Sustainability*, 13, 1318.
 508 <https://doi.org/10.3390/su13031318>
 509 Malhi, Y., Franklin, J., Seddon, N., Solan, M., Turner, M.G., Field, C.B. and Knowlton, N. (2020).
 510 Climate change and ecosystems: threats, opportunities and solutions. *Phil. Trans. R.*
 511 *Soc.B*, 375:1794, <http://doi.org/10.1098/rstb.2019.0104>.
 512 Meine, C. (2018). Biodiversity Conservation. *Reference Module in Earth Systems and*
 513 *Environmental Sciences*, Volume 4, Pages 205-214. Doi: [https://doi.org/10.1016/B978-](https://doi.org/10.1016/B978-0-12-809665-9.10463-X)
 514 [0-12-809665-9.10463-X](https://doi.org/10.1016/B978-0-12-809665-9.10463-X).
 515 Moser, T., Rombke, J., Schallnass, H.J. and Van, G.C.A.M. (2007). The use of the multivariate
 516 Principal Response Curve (PRC) for community level analysis: a case study on the effects
 517 of carbendazim on enchytraeids in Terrestrial Model Ecosystems (TME). *Ecotoxicology*
 518 16: 573–583.

- O'Callaghan, M., Glare, T.R., Burgess, E.P.J. and Malone, L.A. (2005). Effects of plants genetically modified for insect resistance on non-target organisms. *Annu. Rev. Entomol.* 50, 271–292.
- Oksanen, J. (2013). Vegan: ecological diversity. Available at: <http://cran.rproject.org/web/packages/vegan/vignettes/diversity-vegan.pdf>.
- Ortiz, A.M.D., Charlotte, L., Outhwaite, C.D. and Tim, N. (2021). A review of the interactions between biodiversity, agriculture, climate change, and international trade: research and policy priorities. *One Earth*, Volume 4, Issue 1, Pages 88-101, ISSN 2590-3322, <https://doi.org/10.1016/j.oneear.2020.12.008>.
- Pareek, N. (2017).** Climate change impact on soils: adaptation and mitigation. *MOJ Eco Environ Sci.* 2(3):136-139. DOI: [10.15406/mojes.2017.02.00026](https://doi.org/10.15406/mojes.2017.02.00026)
- Pawlak, K. and Kołodziejczak, M. (2020). The Role of Agriculture in Ensuring Food Security in Developing Countries: Considerations in the Context of the Problem of Sustainable Food Production. *Sustainability*, 12, 5488. <https://doi.org/10.3390/su12135488>.
- Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *J Theor Biol* 13: 131–144.
- R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rawat, U.S. and Agarwal, N.K. (2015). Biodiversity: Concept, threats and conservation. *Environment Conservation Journal*, 16(3) 19-28, 2015 ISSN 0972-3099
- References**
- Roe, D., Seddon, N. and Elliott, J. (2019). Biodiversity loss is a development issue: a rapid review of evidence. *IIED Issue Paper. IIED, London*, <http://pubs.iied.org/17636IIED> ISBN 978-1-78431-688-4
- Romeis, J., Meissle, M., Naranjo, S.E., Li, Y. and Bigler, F. (2014). The end of a myth – Bt (Cry1Ab) maize does not harm green lacewings. *Front Plant Sci* 5, 1–10.
- Romeis, J., van Driesche, R.G., Barratt, B.I.P. and Bigler, F. (2008). Insect-resistant transgenic crops and biological control. In: Romeis J, Shelton AM and Kennedy GG, , editors., *Integration of insect-resistant genetically modified crops within IPM programs. Springer Science + Business Media BV.* pp. 87–117.
- Salama, H.S. and Zaki, F.N. (1983). Interaction between *Bacillus thuringiensis* Berliner and the parasites and predators of *Spodoptera littoralis* in Egypt. *Z angew Entomol*, 95: 425–429.
- Schorling, M. and Freier, B. (2006). Six-year monitoring of non-target arthropods in Bt maize (Cry 1Ab) in the European corn borer (*Ostrinia nubilalis*) infestation area Oderbruch (Germany). *J Verbr Lebensm* 1: 106–108.
- Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried, B.D. and Dively, G.P. (2001). Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11937–11942.
- Skendžić, S., Zovko, M., Živković, I.P., Lešić, V. and Lemić, D. (2021). The Impact of Climate Change on Agricultural Insect Pests. *Insects*. 12;12(5):440. doi: [10.3390/insects12050440](https://doi.org/10.3390/insects12050440).
- Smyth, S.J. (2020). The human health benefits from GM crops. *Plant Biotechnol J.* 18(4):887-888. doi: [10.1111/pbi.13261](https://doi.org/10.1111/pbi.13261). Epub 2019 Oct 2. PMID: 31544299; PMCID: PMC7061863.
- Smyth, S.J., McHughen, A., Entine, J., Kershen, D.R. and Parrott, W. (2021). Removing politics from innovations that improve food security. *Transgenic Research*, 30, 601–612. <https://doi.org/10.1007/s11248-021-00261-y>

566 Subedi, B., Poudel, A., Aryal, S. (2023). The impact of climate change on insect pest biology
 567 and ecology: Implications for pest management strategies, crop production, and food
 568 security. *Journal of*
 569 *Agriculture and Food Research*, Volume 14, ISSN 26661543, <https://doi.org/10.1016/j.jafr.2023.100733>.
 570
 571 Tilman, D., Isbell, F. and Cowles, J.M. (2014). Biodiversity and Ecosystem Functioning. *Annual*
 572 *Review of Ecology, Evolution, and Systematics*, Vol. 45:471-493;
 573 <https://doi.org/10.1146/annurev-ecolsys-120213-091917>
 574 Triplehorn, C.A., Johnson, N.F. and Borror, D.J. (2005). Borror and DeLong's Introduction to
 575 the Study of Insects. *Thomson Brooks/Cole, Belmont, CA*.
 576 Vanden-Brink, P.J., den-Besten, P.J., bijde-Vaate, A. and ter-Braak, C.J.F. (2009). Principal
 577 response curves technique for the analysis of multivariate biomonitoring time series.
 578 *Environ Monit Assess* 152: 271–281.
 579 Vasiliev, D. (2022). The Role of Biodiversity in Ecosystem Resilience. *IOP Conference Series*
 580 *Earth and Environmental Science*, 1072(1):012012, DOI:10.1088/1755-
 581 1315/1072/1/012012
 582 Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J. and Watrud, L.S. (2008). *Bt* crop
 583 effects on functional guilds of non-target arthropods: a meta-analysis. *PLoS ONE*, 3:
 584 e2118.
 585 Zhang, B.Y., Chen, M., Zhang, X.F., Luan, H.H. and Tian, Y.C. (2011). Expression of Bt-Cry3A in
 586 transgenic *Populus alba* x *P. glandulosa* and its effects on target and non-target pests
 587 and the arthropod community. *Transgenic Res* 20: 523–532.