January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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The efficacy of genetically modified (GM) corn Bt11 against *Ostrinia furnacalis* (Guenee) and *Helicoverpa armigera* (Hubner)

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Bahagiawati and Diani Damayanti

The Efficacy of Genetically Modified (GM) Corn Bt11 against *Ostrinia furnacalis* (Guenee) and *Helicoverpa armigera* (Hubner)

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Abstract. Corn pests such as corn stem borer *Ostrinia furnacalis* and corn earworm *Helicoverpa armigera* could reduce yield quantitatively and qualitatively. Bt11 is a genetically modified corn that contains the *cry1Ab* gene that has been proven in other countries can control the lepidopteran insect pest of corn. The objective of this experiment was to evaluate the efficacy of Bt11 against *O. furnacalis* and *H. armigera* that are found in Indonesia. The experiments were conducted in a containment facility at ICABIOGRAD using leaf tissue and whole plant assays for *O. furnacalis* and fresh ear assay for *H. armigera*. The experimental design was completely randomized with two treatments, Bt11 and non-Bt11 plants with 20 replicates for leaf tissue assay and fresh ears assay and 16 replicates for whole plant assay. The statistical analysis was a t-test. The results showed that Bt11 is highly efficacious against *O. furnacalis* and *H. armigera*.

INTRODUCTION

Corn is the second important food crop after rice in Indonesia and it is used as food and feed. One of the main problems with yield and grain quality in corn production is the damage caused by one of the key pest of corn, the stem borer. In Europe and the United States, the common corn stem borer species belongs to *Ostrinia nubilalis* [1, 2]. However, in Asia including Indonesia, the stem borer belongs to the species of *O. furnacalis* [3, 4]. The yield reduction from stem borer is about 20–80% [5, 6]. Serious damage occurs when corn stems are heavily infested during the tasseling stage of the plant development, and many stems break from larval burrowing resulting in total loss. Considerable damage is also caused from larval leaf feeding during the whorl stage of plant growth and from larvae boring into young ears [5]. In addition, the earworm *Helicoverpa armigera*, is a pest that is found in the ears [7]. Ear damage results in poor grain quality, making it difficult to market [8, 9].

The common control measure of stem borer and corn earworm is the use of insecticides, but this control technique is not effective because these pests live and grow inside the stems and ears of plants [10]. Currently, the available conventional varieties are not efficacious against these pests [6].

The development of recombinant DNA technology, genetically engineered technology, has opened up great opportunities for research and application in plants. This involves transferring good genes from other organisms into plants, for example, the insecticidal gene of Bt11 was transformed by inserting the *cry 1Ab* gene derived from the bacterium *Bacillus thuringiensis* into the corn plant [11]. Genetic engineering techniques can be used to complement conventional breeding techniques and other insect control techniques [12].

The *cry* genes that are isolated from the soil bacterium *B. thuringiensis*, produce crystalline proteins that kill the insect pests, that include species from the Lepidoptera group [13, 14]. The mode of action of the *cry* genes is, when the insect pest ingests the Cry protein, it enters the digestive tract of the insect and attaches to the receptors in the brush border membrane of the insect's intestine. This causes pores to form resulting in the leakage of the intestine thus, killing the insects. However, these insecticidal proteins are not harmful to nontarget insects (parasites and predators) or other organisms such as birds and other vertebrates [15]. Corn containing *Bt* genes have been widely

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grown and marketed in various countries of the world [16]. One of the *Bt* corns that has been declared safe for food, feed, and the environment in several countries is the Bt11. Bt11 is stacked with another event, e.g. GA21, which is the herbicide tolerant component [17]. Bt11 corn products have been shown to be efficacious against corn stem borer *O. nubilalis* and *O. furnacalis* in other countries [18]. However, this Bt11 has not yet got a variety released permit in Indonesia and has not been tested whether it resistant to stem borer and earworm that present in Indonesia. To get a varietal released permit this Bt11 has to obtain three biosafety certificates (food, feed, and environment). The Bt11 corn was declared safe for food, feed, and environment in Indonesia, in 2011, 2018, and 2020, respectively [19, 20]. The purpose of this study was to evaluate the efficacy Bt11 corn against *O. furnacalis* and *H. armigera* that are found in Indonesia.

MATERIALS AND METHODS

Materials

The studies were conducted at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) in Indonesia. Test plants were Bt11 and the non-*Bt* corn. The seeds were obtained from PT Syngenta Indonesia. The insects *O. furnacalis* and *H. armigera* were collected from sweet corn fields near Bogor.

Insect Rearing

Larvae and pupae of stem borer and corn earworm from the field were reared in the laboratory. The newly hatched larvae were kept in jars filled with artificial diet until they became pupae. The pupa was moved into pupa jar until it became a moth. The moths were transferred into adult jars that have been provided with honey and a piece of paper for egg laying. Eggs that were laid on the paper were stored in a container and allowed to hatch, and the neonates were used for the experiments [21, 22].

Evaluation of the Efficacy of Bt11 Corn against *O. furnacalis*

Leaf Tissue Assay

The youngest leaves of Bt11 corn and the non-*Bt* corn were taken from plants grown in the greenhouse at 2, 4, 6, 8 weeks after planting (WAP). Leaves were cut into 5 cm long then placed into a Petri dish with a piece of filter paper moistened with distilled water. Ten neonates of *O. furnacalis* larvae were infested onto the leaf in the Petri dish using a small brush, then the Petri dish was tightly closed with its lid. The Petri dishes were then placed in a room at temperature of 25°C. Each treatment had 20 replications. A leaf in one Petri dish represented one replicate [22].

Data Collection

On the 7^{th} days after larval infestation, larval mortality and leaf damage ratings were recorded. Leaf damage rating scales of class 1–9 was based on Guthrie *et al*. 1960 [23] as follows: Class 1 = no visible damage or there are a few small holes in the leaves, Class 2 = some damage in the form of a small hole in the leaf, Class 3 = a small amount of damage in the form of a large hole in the leaves, Class 4 = some damage in the form of large holes extending in the leaves, Class 5 = a lot of damage in the form of large holes extending in the leaves, Class 6 = a lot of damage in the form of a large hole with a length of 2.5 cm, Class 7 = damage in the form of a large hole in 1/5 of the leaf, Class 8 = leaf damage in the form of large holes in 2/5 of the leaves, and Class 9 = leaf damage in the form of large holes in all parts of the leaf. Meanwhile, scoring scale 1–9 were divided into three categories of resistance as follows: 1–3 = highly resistant, 4–6 = moderately resistant, and 7–9 = susceptible.

Whole Plant Assay

Corn seeds were planted in a plastic pot (diameter 30 cm) in the greenhouse, with one seed per pot. The pots were divided into two groups of 16 plants in one group of Bt11 corn and 16 pots of the non-*Bt* in the second group. Each plant was infested with five egg masses of *O. furnacalis* at 4 and 12 WAP [22].

Data Collection

Plant damage caused by *O. furnacalis* was assessed three weeks after infestation using the rating scale by Guthrie *et al*. [23]. The number of holes per stem and the length of tunnel in the stem were recorded. The stems with holes were dissected longitudinally and the tunnelling lengths were measured in centimeter. The design of this experiment was a completely randomized design with two treatments (Bt11 and non-*Bt* corn). Each plant represents one replication, and there were 16 replications for each treatment.

Statistical Analysis

A t-test statistical from the SPSS 10 software was used to analyze the data.

Evaluation of the Efficacy of Bt11 Corn against *H. armigera*

Fresh Ear Assay

Corn ears, were harvested from corn grown in the greenhouse at 8 WAP cleaned and then placed in plastic cups. On ear for one plastic cup. Each corn ear was covered with moistened cotton and coated with parafilm to keep the corn ear fresh during the test. Ten neonates were infested into the silk on the ear using a small brush. The plastic cup was then covered with another plastic cup (Fig. 3). The plastic cups containing corn ear, and larvae were stored in a room at 25°C. Each plastic cup, represented one replicate and this experiment had 16 replications. The design of this experiment was a completely randomized design with two treatments (Bt11 and non-*Bt*).

Data Collection

At two weeks after infestation, the percent larval mortality and length of tunnel in the ears were measured in centimeter. Ears damage rating scale were recorded using rating scale by Widstrom (1967) [24] as mentioned in Table 1.

TABLE 1. Ears damage rating scale recorded using rating scale by Widstrom (1967) [24].

Resistance category	Score	Description
Resistance (0–2)	0	No visible damage on corn ear/cob
	1	Damage only detected on hair/silk of corn cob
	2	Larvae penetration (from the tip of the cob) up to 1 cm
Moderate resistance (3)	3	Larvae penetration up to 2 cm from tip of the cob
Susceptible (4.....n.)	≥4	Larvae penetration more than 3 cm long from the tip of the cob

Statistical Analysis

A t-test statistical from the SPSS 10 software was used to analyze the data.

RESULTS AND DISCUSSIONS

Evaluation of the Efficacy of Bt11 Corn against *O. furnacalis*

Leaf Tissue Assay

The result of leaf tissue assay showed that, the corn leaves from the Bt11 plant were highly resistant to *O. furnacalis*. Bt11 leaves had low leaf damage score and a high larval mortality. On the other hand, the non-*Bt* leaves showed a high leaf damage score and a low larval mortality rate. This result was consistent for all tested leaves taken at 2, 4, 6, 8 WAP (Table 2).

TABLE 2. The leaf damage ratings and percent larval mortality of *Ostrinia furnacalis* on Bt11 corn and non-*Bt* corn at 7 days after infestation.

Event	Plant age								Level of resistance
	2 WAP		4 WAP		6 WAP		8 WAP		
	SLD	LM (%)	SLD	LM (%)	SLD	LM (%)	SLD	LM (%)	
Bt11	1.50	100	1.10	100	1.50	100	1.50	100	Highly resistance
Non-*Bt*	7.65	0	8.85	0	9.00	0	7.65	0	Susceptible

WAP = weeks after planting, SLD = score of leaf damage, LM = percentage of larval mortality.

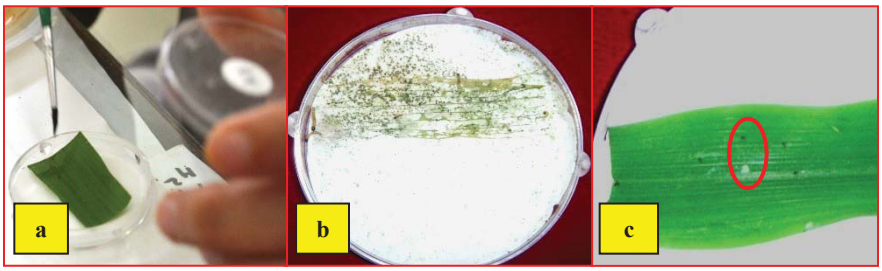

FIGURE 1. Leaf tissue assay. (a) Infestation of *Ostrinia furnacalis* larvae. (b) Non-*Bt* leaf had many large feeding marks and the life larvae developed into late instars. (c) Bt11 leaf, red circle shows dead larvae of *O. furnacalis* and larvae feeding marks. Bt11 leaves had very few small feeding marks compared to non-*Bt* leaves.

Whole Plant Assay at 4 WAP (Vegetative Stage)

The number of holes and the tunneling length was significantly different for Bt11 and its non-*Bt* corn at ($P<0.05$). Bt11 corn had no hole, while in the non-*Bt*, had an average of 2.4 holes per stem (Table 3). The tunnel length of the non-*Bt* corn was 4.13 cm. Therefore, it was concluded that the Bt11 corn plant was resistant to stem borer pests.

TABLE 3. The average number of feeding holes and tunneling length per stem by *Ostrinia furnacalis* at the vegetative stage for Bt11 and non-*Bt* corn plants.

Event	Average number of hole per stem	Average length of tunnel (cm)	Level of resistance
Bt11	0 b	0 b	Highly resistance
Non-*Bt*	2.40 a	4.13 a	Susceptible

Whole Plant Assay at 12 WAP (Generative Stage)

The number of feeding holes and the tunneling length were significantly different for Bt11 and non-*Bt* corn at (P<0.05). There was no feeding hole on the Bt11 corn stems indicating that the Bt11 corn was resistant to *O. furnacalis* during the generative stage (Table 4). The average number of holes in non-*Bt* stems were 3.5 holes and the average length of tunnel was 4.71 cm. This means that the stem borer feed well on non-*Bt*. The stem borer fed on the stem by boring the stem and producing tunnel, and at the final stage when the larvae became adult, the adult insect will exit from the holes that were produced. From average number of hole that found in the non-*Bt* stem which was 3.5 it was indicated that the number of stem borer per stem was more than one.

TABLE 4. The average number of feeding holes and tunneling length per stem by *Ostrinia furnacalis* at the generative stage of Bt11 and non-*Bt* corn plants.

Event	Average number of hole per stem	Average length of tunnel (cm)	Level of resistance
Bt11	0 b	0 b	Highly resistance
Non-*Bt*	3.50 a	4.71 a	Susceptible

The means within the column are significantly different at (P<0.05) by t-test.

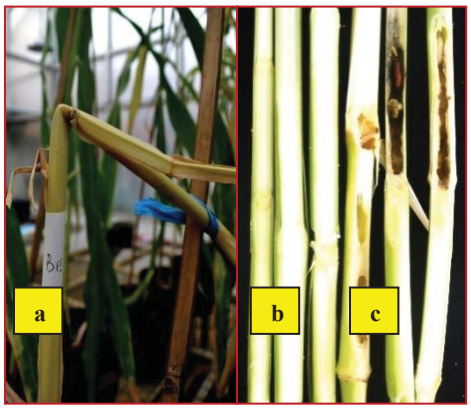

FIGURE 2. Comparison of generative stage (15 WAP) resistance of Bt11 and the non-*Bt* corn for resistance to *Ostrinia furnacalis*. (a) Non-*Bt* corn plants attacked by *O. furnacalis*. (b) Bt11 corn stems with no damage by *O. furnacalis*. (c) Non-*Bt* corn stems varieties with damage by *O. furnacalis*.

Evaluation of the Efficacy of Bt11 Corn against *H. armigera*

Fresh Ears Assay

Table 5 shows that the t-test was significant for percent larval mortality and tunnel length for Bt11 and the non-*Bt* corn at (P<0.05). The tunnel length of the Bt11 corn and the non-*Bt* corn was 0.34 cm and 4.67 cm, respectively. The percent larval mortality of *H. armigera* was 100% and 6% for the Bt11 and non-*Bt* corn, respectively. Therefore, it was concluded that Bt11 corn ears were resistant to *H. armigera*.

TABLE 5. Mortality of *Helicoverpa armigera* and ear tunnel length of *H. armigera* of Bt11 and non-*Bt* corn.

Event	Larvae mortality (%)	Tunnel length (cm)	Level of resistance
Bt11	100 a	0.34 a	Resistance
Non-*Bt*	6.00 b	4.67 b	Susceptible

The means within the column were significantly different at (P<0.05) by t-test.

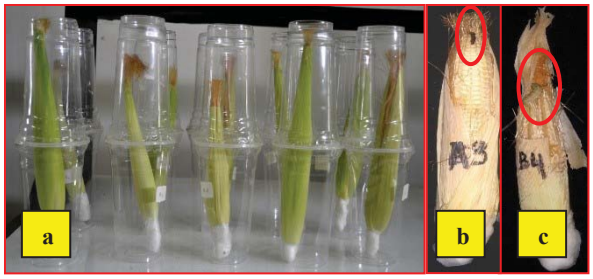

FIGURE 3. Resistance of Bt11 corn ears to *Helicoverpa armigera* in laboratory. (a) The fresh ear assay performance in laboratory. (b) Bt11 corn, red circle shows dead larvae. (c) Non-*Bt* corn, red circle shows live larvae.

Results of this study show that, the B11 corn is resistant against the two major insect pests of corn in Indonesia, namely *O. furnacalis* and *H. armigera*. He *et al*. 2003 [18] showed that Bt11-containing maize was resistant to *O. furnacalis*. In order to increase the effectiveness of Bt11 corn, this line was crossed with herbicide tolerant GM corn, the progeny of the cross known as stacked corn. Right now, the stacked *Bt* corn is grown on a large scale by farmers than single trait corn [16].

Transgenic corn stacked with Bt11 event have been grown in Philippines and Vietnam. In the Philippines, Bt11 received a commercialization permit in 2003 and was followed by the approval of other single and stacked events containing Bt11 and have been proven to be able to control the stemborer [25]. Farmers in the Philippines in 2003, planted approximately 20,000 ha of *Bt* corn, and the production was 41–60% higher than the production of corn farmers who did not grow *Bt* corn. This resulted in an increase in average farmer net income of 34% [26]. In 2019, the area of *Bt* maize with stacked traits (pest resistant and herbicide tolerant) that was planted globally reached 50.8 million ha [16].

Farmers in Vietnam started planting *Bt* corn in 2015, and in 2018 the area planted to *Bt* corn was 49,000 ha. The survey results from Brookes and Dinh [27] showed that, *Bt* corn production increased by 30.4% and reduced production costs between USD 26.47–31.30 per ha. The insecticide used to control maize pests decreased by 78% per ha.

In Indonesia Bt11 received certificate for food safety in 2011 from the Indonesia National Agency for Drugs and Food Control, for feed safety certificate from the Ministry of Agriculture in 2018, and for environmental safety certificate from the Ministry of Environment and Forestry in 2020 [28]. Based on the results of this study and also the studies conducted in the Philippines and Vietnam, it is expected that Bt11 corn and its stacked products will control *O. furnacalis* and *H. armigera* in Indonesia.

CONCLUSION

Results of leaf assay and whole plant assay both on vegetative and generative stages of plant development showed that Bt11 was resistant to *O. furnacalis.* In addition, the fresh ear assay also showed that Bt11 was resistant to *H. armigera*. This study indicates that Bt11 was efficacious against *O. furnacalis* and *H. armigera* that are found in Indonesia and can be used to manage these insects and hopefully would get a release permit to be used by Indonesian farmers.

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