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Comparison between the performance of genetically modified and conventional maize hybrids in Brazil

Leandro Nogueira Ramos[1]*, Nara Oliveira Silva Souza[2], Maurício Kobiraki[1]

[1]DuPont Pioneer, Cx. Postal 08283, CEP 73301-970, Planaltina, DF, Brazil
[2]University of Brasília, FAV, Cx. Postal 04508, CEP 70910-900, Brasília, DF, Brazil

***Corresponding author:** zleandro.ramos@gmail.com

Abstract

This study evaluates the yield performance of five maize hybrids (HP2251, HP5253, HP6490, HP8761 and HP0297). The aim of this work was to compare a non-transgenic (base genetics), a transgenic (*Bt*) with TC1507 and a transgenic (*Bt*) with MON810+TC1507 for each hybrid. The experiment was conducted in farms during 2014-2015 season, with natural infestation of fall armyworm and controlled by spraying insecticides. Three locations were planted in the Federal District, another two in Minas Gerais and three in Goiás (Brazil). The experimental design was a complete randomized block of 5 x 3 factorial arrangement in 8 locations, 5 hybrids and 3 versions, with two replicates. The plot size was four rows of five meters. For grain yield, data on weight was converted to kg.ha^{-1} and moisture was standardized to 14%. Harvest data was submitted to statistical analysis using ASReml program to obtain yield predictions of genotypic effects. The estimation of variance components and genotypic parameters were obtained by Restrict Maximum Likelihood process. There were no significant differences when the treatments were analysed for the presence or absence of transgenic genes. The yield differences in the hybrids were due to the adaptability of those genotypes to the Brazil central high lands and not necessarily to the insertion of *Bt* genes. The transgenic insertions were not a determinant factor for yield reduction, indicating a specific interaction between genotypes and *Bt* events for yield. Therefore, a new transgenic hybrid always must be compared to its conventional counterpart before release decision.

Keywords: genetic resistance, *Spodoptera frugiperda,* transgenic versions, yield performance, *Zea mays.*
Abbreviations: BLUP_Best linear unbiased prediction; *Bt_Bacillus thuringiensis*; C_Conventinal; H_event TC1507; REML_Restricted maximum likelihood; YH_ events MON 810 and TC1507.

Introduction

Brazil is the third maize (*Zea mays* L.) producing country, after the US and China, producing approximately 93 million tons. Brazilian maize exports account for 18% of total maize exports in the world, behind the US only. Maize production was estimated at a record 97.7 million tons in the 2016/2017 harvest, 46% increase from 2016 to 2017 based on the expanded area and improved yields. Brazil's record maize output has led to record exports estimated at 35 million tons in 2016/2017, more than doubling the export volume of 2015 (USDA 2017). The summer maize area planted in the 2016/17 harvest was 5.4 million hectares, while the area of off-season crop (second harvest of 2015) was 12.1 million hectares. These areas place maize as the second largest crop in Brazil, behind soybeans only (*Glycine max* L.), with an estimate of more than 34 million hectares planted in 2016/17 (Conab 2017).

Maize has a broad scope in Brazil. In the southern and southeastern regions, maize is usually cultivated in the first season, while in the South and Midwest; it is grown in the off-season. However, maize is predominantly grown in tropical regions, where there is a high incidence of pests. The main maize pest is the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). When not properly managed with chemical, biological and/or genetic controls, this pest causes variable losses depending on the phenological stage of the crop and period of the year. Several authors reported losses of up to 34% due to this factor (Cruz and Turpin, 1983, Cruz 1995, Figueiredo et al., 2006, Werle et al., 2011).

Some biological aspects of *Spodoptera frugiperda* favor the occurrence of severe infestations, which include high polyphagia, high reproductive capacity, ease of dispersion when adults and high number of cycles, which may reach eight per crop year. (Bernardi et al 2015). Those factors make crop difficult and considerably overload the control measures.

In addition to the aspects involved in pest biology, the area planted in the off season currently comprises approximately 59% of the total area of maize grown in Brazil. The other 41% are grown in the summer. Consequently, the maize planting window in Brazil is long, and maize is currently in different growth stages during the twelve months of the year (Conab 2017). This increases the pressure of pests in their adult stage; which, through migration, infests the maize fields in the early stages.

Genetic breeding is widely used for different pests and crops in different parts of the world as a preventive, practical and effective option for insect control. In Brazil, the main genetic technique for the control of *Spodoptera frugiperda* is the hybrid of transgenic maize. It expresses the *Bt* gene, cloned from the bacterium *Bacillus thuringiensis* (Berliner), which encodes a protein toxic to several insects (Boulder 1993, Waquil et al., 2002).

In maize producing countries, 231 events were approved and 202 were approved specifically for the control of insects in maize. The Brazilian National Biosafety Committee (CTNBio) approved 39 commercial transgenic events related to maize in Brazil. Sixteen events were approved for resistance to lepidopteran insects, 6 for coleopterans and 3 for multiple insect resistances (ISAAA 2015). The MON810 event, YieldGard technology, was effective in controlling *Spodoptera frugiperda* and *Helicoverpa zea* in southern Georgia in maize hybrids in 1998 (Buntin et al., 2001).

Maize hybrids expressing the Cry1Ab protein (event MON810) or expressing the Cry1F protein (event TC1507), also tested in the USA, have been effective in the control of *Spodoptera frugiperda* and *Helicoverpa zea* in 2006 and 2007 (Buntin, 2008). Siebert et al., (2008) also obtained high control levels of *Spodoptera frugiperda* in transgenic maize hybrids expressing Cry1F in the southeast of the USA.

The Cry1F protein expressed in maize by the TC1507 event was released commercially in 2008 and marketed from 2009. It has been used successfully in Brazil for a few years. However, neonate larvae of *Spodoptera frugiperda* collected in TC1507 transgenic maize fields infesting the west of Bahia in 2011 were able to survive in Cry1F maize plants under laboratory conditions and subsequently produce fertile adults (Farias et al., 2014). This indicates that there is dynamism in the effectiveness of such transgenic events under crop conditions, planting dates and size of maize areas in Brazil. Therefore, other events expressing different *Bt* proteins are necessary for controlling *Spodoptera frugiperda*.

Studies on transgenic maize hybrids focus primarily on efficacy tests, both of insecticidal proteins and transgenic plants, and on the monitoring efficacy of a particular event over the years (harvests). However, it is important to compare how a similar performance of transgenic hybrid versions stands in relation to the corresponding versions of conventional hybrids. Unlike efficacy tests, performance tests should be performed on optimal planting, management, cultivation conditions and especially with insecticide applications in all treatments. Therefore, this study investigated the production yield of transgenic maize hybrids and their conventional counterparts conducted in grain production farms in 2014-2015 harvest to compare their productive potential.

Results and Discussion

Grain yield performance

Table 1 shows no differences on performance related to presence or absence of the *Bt* technology among the 15 types of maize hybrids (5 hybrids x 3 versions - non GMO despite being conventional or containing the TC1507 (H) or TC1507 + MON810 (YH) transgenic genes). The yield classification of hybrids shows no tendency to form groups because of such technology.

There was a significant difference in performance among the studied hybrids when considering the average productivity of the three versions of them. The HP2251 was the most productive (11,421 kg ha^{-1}) and the HP0291 was the least productive (10,545 kg ha^{-1}), representing 7.8% decreased productivity in relation to HP2251 (Table 2). Regardless of the aggregate technology, these data indicate a higher or lower productivity according to the adaptability of these genetic materials in the centre-west region of Brazil, and not necessarily the insertion of genes with a *Bt* technology.

Grain yield performance between transgenic and non-transgenic hybrids

There was 1.29% increase in productivity in the YH versions compared to conventional versions and 0.61% increase in the H version when considering the average productivity of the five hybrids together. Therefore, the insertion of genetic modified events H and YH did not affect the productivity of hybrids when they were analysed together (Figure 1).

Bortoloto and Silva (2009) found no differences between the hybrids 30F80Y and 30K75Y containing the Y technology and the conventional hybrids 30F80 and 30K75 regarding productivity and other agronomic traits.

Moraes et al., (2015) also found the conventional version and its isogenic of maize genotypes did not differ for grain yield.

In addition, Holland and Goodman (2003) evidenced that the use of the *Bt* technology did not result in increased productivity, with no differences between conventional and transgenic isogenic genotypes.

Table 3 shows the yield of the five hybrids in each version. For HP0297, the version HP0297YH achieved the highest productivity. However, the HP0297H version produced 4.14% less than the conventional version HP0297C. This also occurred to the hybrid HP5253, which produced 3.72% less than the conventional version HP5253C. On the other hand, HP5253YH produced 0.48% more than the conventional version HP5253C. Differences are irrelevant for HP2251. Both H and YH versions presented yield close to the conventional version.

When each hybrid is individually studied, there is discrepancy in productivity differences between versions (Table 3). This indicates a possible genotype/genes interaction of *Bt* proteins.

Regarding the hybrid HP6490, there was a considerable productivity increase in its transgenic versions compared to the conventional version HP6490C. The version HP6490H achieved 17.1% higher production than the conventional version, and HP6490YH reached a 14.5% higher production. It is important to consider that the 2.1% difference between the H and YH versions is not limiting for using the two sources of resistance to *Spodoptera frugiperda* in relation to yield. As for the hybrid HP8761, the two transgenic versions provided productions below the conventional version, with decreases of 4.5% (H) and 6.8% (YH), compromising the future inclusion of these versions in the market because they are not competitive with the conventional version in terms of yield (Table 3).

Table1. Ranking of conventional maize hybrids (C), transgenic version containing the TC1507 (H) event and transgenic version containing the TC1507 + MON810 (YH) events in relation to grain yield achieved by μ, Best linear unbiased prediction (BLUP) analyzed by the statistical software ASReml. Brasília-DF, 2015.

Ranking	Hybrids	Versions	μ (BLUP-kg.ha^{-1})	** Difference of yield performance (%)
1º	HP2251H	H	11,497	
2º	HP2251C	*C	11,440	-0.5
3º	HP6490H	H	11,367	-0.6
4º	HP2251YH	YH	11,326	-0.4
5º	HP5253YH	YH	11,218	-1.0
6º	HP5253C	*C	11,164	-0.5
7º	HP6490YH	YH	11,118	-0.4
8º	HP8761C	*C	11,081	-0.3
9º	HP0297YH	YH	10,759	-2.9
10º	HP5253H	H	10,749	-0.1
11º	HP0297C	*C	10,659	-0.8
12º	HP8761H	H	10,587	-0.7
13º	HP8761YH	YH	10,328	-2.4
14º	HP0297H	H	10,218	-1.1
15º	HP6490C	*C	9,706	-5.0

* - Conventional non-transgenic version. ** - Percentage difference (%) referring to the difference of productivity within the ranking of the 15 hybrids tested.

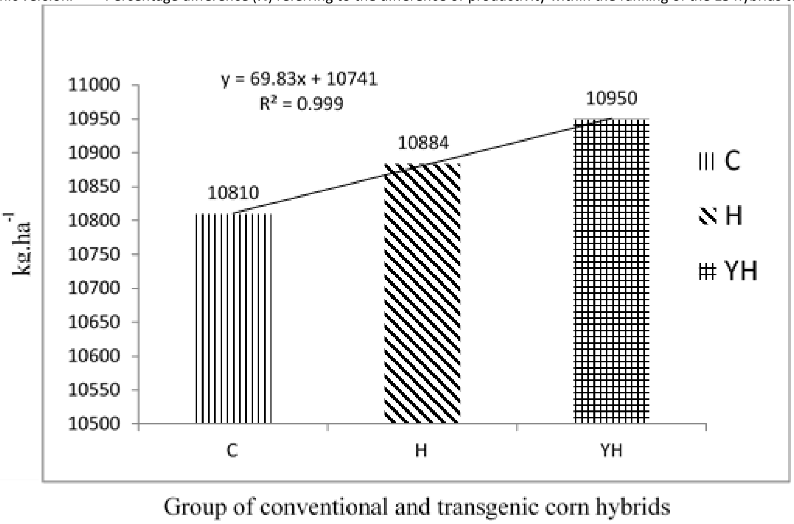

Fig 1. Means of conventional maize hybrids, transgenic version containing the TC1507 (H) event and transgenic version containing the TC1507 + MON810 (YH) events in relation to grain yield achieved by Best linear unbiased prediction (μ-BLUP) analyzed by the statistical software ASReml. Brasília-DF, 2015.

Table 2. Classification of maize hybrids in relation to grain yield achieved by μ (BLUP) considering the conventional version, transgenic versions containing the TC1507 (H) event and transgenic version containing the TC1507 + MON810 (YH) events analyzed by the statistical software ASReml. Brasília-DF, 2015.

Ranking	Hybrid	μ *(BLUP-kg.ha^{-1})	** Difference of yield performance (%)
1º	HP2251	11,421	
2º	HP5253	11,044	-3.3
3º	HP6490	10,730	-2.8
4º	HP8761	10,665	-0.6
5º	HP0297	10,545	-1.1

* - Average productivity of conventional, H and YH versions;
** Percentage difference (%) referring to the difference of productivity within the ranking of the 5 groups of hybrids.

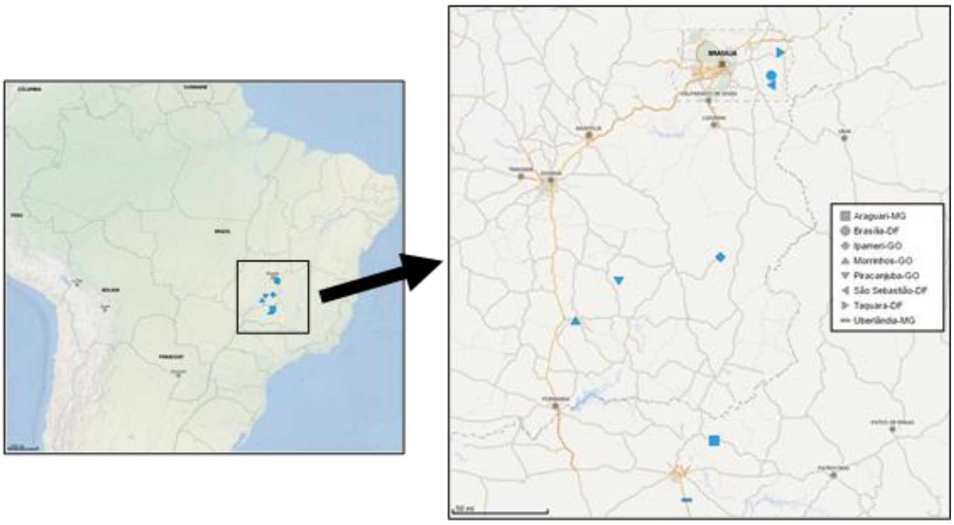

Fig 2. Eight farms in eight cities (Araguari, Brasília, Ipameri, Morrinhos, Piracanjuba, São Sebastião, Taquara e Uberlândia) The forms indicate where the experiments were conducted

Table 3. Percentage differences of yield of maize hybrids obtained by μ (BLUP) considering the conventional version, transgenic version containing the TC1507 (H) event and transgenic version containing the TC1507 + MON810 (YH) events analyzed by the statistical software ASReml. Brasília-DF, 2015.

Hybrid	Transgenes	μ (BLUP-kg.ha^{-1})	*H x C	**YH x C
HP0297	non-transgenic	10,659		
HP0297H	Cry1F	10,218	- 4.1	
HP0297YH	Cry1F + Cry1Ab	10,759		0.9
HP5253	non-transgenic	11,440		
HP5253H	Cry1F	11,497	- 3.7	
HP5253YH	Cry1F + Cry1Ab	11,326		0.5
HP2251	non-transgenic	11,164		
HP2251H	Cry1F	10,749	0.5	
HP2251YH	Cry1F + Cry1Ab	11,218		- 1.0
HP6490	non-transgenic	9,706		
HP6490H	Cry1F	11,367	17.1	
HP6490YH	Cry1F + Cry1Ab	11,118		14.5
HP8761	non-transgenic	11,081		
HP8761H	Cry1F	10,587	- 4.4	
HP8761YH	Cry1F + Cry1Ab	10,328		- 6.8

* - Percentage difference (%) between the versions H and C
** - Percentage difference (%) between the versions YH and C

Table 4. Geographical locations and altitude at sea level, dates of planting and harvest dates of the maize experiment. Brasília-DF, 2015.

Locations	Latitude	Longitude	Altitude (m)	Planting	Harvest
Brasília-DF	-15.84961	-47.49536	1,053	11/01/2014	04/11/2015
São Sebastião-DF	-15.92563	-47.49558	1,087	10/31/2014	04/12/2015
Taquara-DF	-15.67230	-47.42108	1,093	11/06/2014	04/13/2015
Ipameri-GO	-17.25313	-47.90713	1,172	11/11/2014	04/14/2015
Morrinhos-GO	-17.73603	-49.06414	758	11/09/2014	04/15/2015
Piracanjuba-GO	-17.43679	-48.721512	733	11/26/2014	04/16/2015
Uberlândia-MG	-19.10682	-48.174073	813	11/11/2014	04/17/2015
Araguari-MG	-18.65594	-47.955334	883	11/18/2014	04/18/2015

Farinelli and Cerveira Júnior (2014) also detected grain yield increase of the hybrid AG8088 VTPRO, a transgenic with two *Bt* proteins resistant to *Spodoptera frugiperda*, when compared to the conventional AG 8088, which increased plant density per area. Lourenção & Fernandes (2013) tested the effectiveness of the *Bt* hybrids Cry1Ab and Cry1F on *Spodoptera frugiperda* and their performance comparing with their conventional isolines, obtaining higher yields with transgenic versions in some cases.

Materials and Methods

Plant materials

Maize hybrids adapted to the central-north region of Brazil, with high productive potential and different maturity cycles were selected to determine the effect of the transgenic events insertion on grain yield. Another relevant factor was the good tolerance of these hybrids to the main foliar diseases, stalk rot and ear rot of maize, which is frequent in this region.

Treatments

Five maize hybrids were evaluated: HP2251, HP5253, HP6490, HP8761 and HP0297. Each hybrid had three different versions: conventional (C), expressing the protein Cry 1F (TC1507 event) (H), and another transgenic version expressing two *Bt* proteins, Cry 1Ab and Cry 1F (MON810 and TC1507 events) (YH). The treatments were classified by grain yield where the first one was the most productive and the fifteenth was the least productive.

Conduction of study

The experiment was conducted in commercial fields during the agricultural year 2014-2015 at eight farms in three states of Brazil (Figure 2), three farms in the Federal District, two in Minas Gerais and other three in Goiás, as described in Table 4. These farms were chosen for their cerrado biome with altitudes above 700 meters. At all farms, plantations occurred directly over the remaining straws of previous crops. To ensure that the yield effect would not be influenced by productivity reduction, insecticides were applied to control the caterpillars that naturally infest the experiment. Three applications of insecticides were performed. Methomyl was applied to V2/V3 (800 $mL.ha^{-1}$); Spinosad was applied to V5/V6 (80 $mL.ha^{-1}$); and Clorantraniliprole was applied to V10 (110 $mL.ha^{-1}$). These applications were made to control infestation by *Spodoptera frugiperda* larvae directed to conventional treatments.

Experimental design

The experimental design was randomized blocks with two replications, consisting of four rows of five meters per plot. Only the two central lines were considered useful plots. The row spacing was 0.75 m. Planting was carried out mechanically with a vacuum planter set to sow 30 seeds every five meters. A double 5 x 3 factorial design was used: 5 hybrids and 3 versions. The experiment was planted in 8 farms. Plots were thinned when plants were at the V4 vegetative stage (four developed leaves). Twenty-three plants remained in each row, and a 0.7m-corridor distinguished plots following a same planting direction. The population throughout the test was kept stable after thinning with approximately 65.000 plants per hectare.

Traits measured

A grain yield evaluation was performed in the plot and estimated in $kg.ha^{-1}$. The harvest was mechanically in a 6.45 m^2 useful area using a specific harvester for four-line experiments. Production and grain humidity data at harvest were stored in on-board computer. Humidity was standardized at 14% to estimate the production per plot.

Statistical analysis

The Mixed Model methodology was used to obtain the Best Linear Unbiased Prediction (BLUP) of genotypic effects, and the process of Restricted Maximum Likelihood (REML) was used for the estimation of variance components and genotypic parameters. This method estimates values across distinct locations. Three commercial hybrids recommended for summer planting in the study areas hybrids, 30F53H, P3646H and P3862H, were used as control treatments. They showed different maturity stages. Controls were used for balancing data, being arranged diagonally in each test. Harvest data were extrapolated to $kg.ha^{-1}$ and subjected to statistical analysis using the software ASReml. This method allows the evaluation of unbalanced experiment testing and may be used for both allogamous and autogamous plants with a mixed reproductive system (Garcia & Nogueira 2005, Resende 2016).

Conclusion

Transgenic events TC1507 and TC1507 + MON810 were not the only determining factors for yield decrease; thus indicating specific interaction between genotypes and *Bt* events influencing the productivity of grain yield of maize. Therefore, it is essential to validate any new version to be released commercially for each maize hybrid regarding its productivity and its agronomic performance. It is extremely important to evaluate direct productivity components such as kernel rows, dimensions and weight of ear and grain. Indirect productivity components such as disease resistance and root volume can also reduce grain yield or even grains quality. The crucial point is to ensure that a new version of transgenic maize hybrid is equal to or better than its previous conventional or transgenic versions validated in terms of agronomic characteristics and yield.

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