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Transgenic events interference on maize morphological and productive attributes

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Abstract

Genetically modified plants have high potential for use and benefits; they are dynamic and tend to enable more sustainable agricultural management alternatives as scientific and technical information are applied. However, little is known about the effects of genetic modifications to induce resistance to herbicides and insect attacks on agronomic characteristics of maize plants. The objective of this study was to evaluate the effects of transgenic events on morphological and productive characteristics of maize hybrids. A field experiment was conducted in two crop seasons, using conventional and transgenic isogenic hybrids. The variables analyzed were plant height, ear height, stem diameter, and grain yield. The genetic modifications used in the evaluated transgenic hybrids affected the morphological and productive characteristics of the maize plants. The transgenic hybrids presented 5% higher plant height and 10% higher grain yield than the conventional hybrids.

Keywords: Cry1Ab protein. PAT enzyme. EPSPS enzyme. Grain yield. Environment-genotype interaction.

Introduction

Maize (*Zea mays* L.) is the most produced cereal in the world. However, the production of this cereal may be compromised by several abiotic and biotic factors. The presence of weeds and attack of insect pests are among biotic factors that can result in serious damages to crop yield and, consequently, losses to the rural producer (KOZLOWSKI, 2002; FERNANDES; CARNEIRO, 2006).

Weed competition can reduce the profits of maize crops in 12% to 100% depending on their species and infestation level, soil type, climatic conditions, and phenological stage of the crop (ALMEIDA, 1981; CONSTANTIN; OLIVEIRA, 2005). Pest insects, such as the maize armyworm (*Spodoptera frugiperda* (J.E. Smith, 1797)), cause high losses on maize yield and quality and are difficulty to control (CARVALHO, 1982; WAQUIL et al*.*, 1982; BARROS et al*.*, 2010). In addition, the misuse of herbicides and insecticides contributes to the emergence of resistant plants and insects, which makes phytosanitary control even more difficult and expensive.

In this context, biotechnological techniques have been applied, with the development of genetically modified maize plants that are resistant to herbicides and insect attacks, as a protective alternative to minimize losses caused by these factors (CIB, 2013; CTNBio, 2009). Genes introduced into maize genotypes encoding the expression of the insecticidal Bt protein are effective in controlling

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lepidopterans such as *S. frugiperda*, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and *Diatraea saccharalis* (HUANG et al., 2002). Genes that express the 5-enolpyruvylchiquimate-3-phosphate synthase (EPSPS) enzyme, which are isolated from the *Agrobacterium tumefaciens* bacterium, are introduced into soybean, maize, and cotton genotypes for weed control; these genes make the plant tolerant to the glyphosate herbicide (SPENCER et al., 2000). Thus, it is possible to use broad spectrum herbicides at post-emergence, with low phytotoxicity to crops (BARRY et al., 1992; PADGETTE et al., 1995).

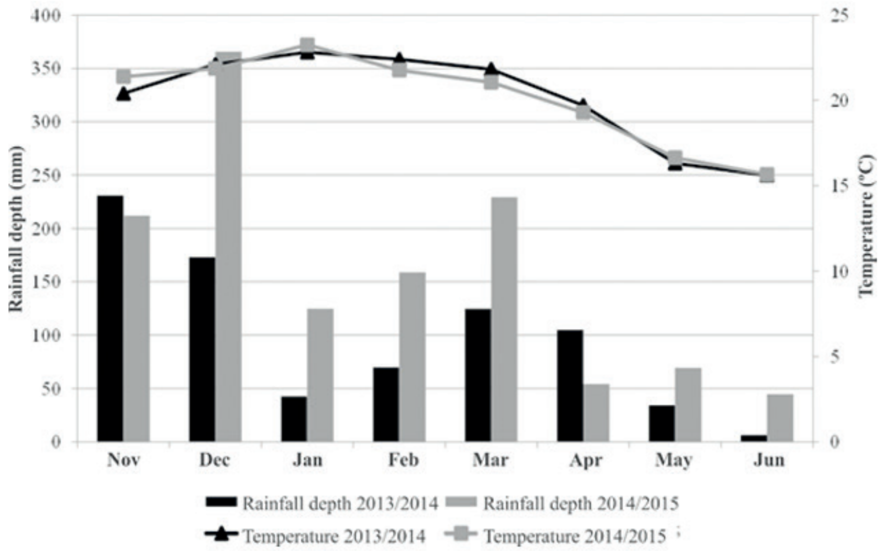
Genetically modified plants have high potential for use and benefits; they are dynamic and tend to enable more sustainable agricultural management alternatives as scientific and technical information are applied. However, little is known about effects of genetic modifications to induce resistance to herbicides and insect attacks on agronomic characteristics of maize plants. Thus, improving the understanding of the effect of these genetic modifications on morphological and productive characteristics of maize hybrids is important to improve cultural practices and phytosanitary managements for this crop species.

The objective of this study was to evaluate the effects of transgenic events on morphological and productive characteristics of maize hybrids. A field experiment was conducted in two crop seasons using conventional and transgenic isogenic hybrids.

Material and methods

The experiment was conducted in two crop seasons (2013-2014 and 2014-2015) at the experimental area of the Federal Institute of Education, Science and Technology of South of Minas Gerais (IFSULDEMINAS), in Inconfidentes, MG, Brazil (22º18'47''S; 46º19'54,9''W; and 940 m of altitude). The soil of the area was classified as eutrophic Red Yellow Latosol (Oxisol) and it had been cultivated with maize for the last four crop seasons. The region is under a Cwb, dry-winter subtropical highland climate, according to the Koppen classification, with average annual temperature of 19.3ºC and average annual rainfall depths of 1.411 mm (BRASIL, 1992; FAO, 1985). The average rainfall depths and temperatures during the study periods are presented in Figure 1.

Figure 1 – Average monthly rainfall depths and temperatures during the study periods (2013-2014 and 2014-2015 crop seasons). Data collected from the weather station at IFSULDEMINAS, Inconfidentes *Campus*.



Source: Prepared by the authors based on data from IFSULDEMINAS - Inconfidentes *Campus* weather station (2018).

The soil chemical attributes of the experimental areas were analyzed before the beginning of the experiment using samples from the 0 to 0.2 m layer. The results showed a pH of 5.3; 3.97 g dm^{-3} of organic matter; 13.25 mg dm^{-3} of P; 85.7 mg dm^{-3} of K; 2.39 cmol dm^{-3} of Ca; 0.57 cmol dm^{-3} of Mg; 4.95 $cmol_c$ dm^{-3} of H+Al at pH 7.0; and base saturation of 39.09%. Subsequently, liming and fertilization were performed according to recommendations of the state of Minas Gerais for maize crops (RIBEIRO et al., 1999).

The experiment was conducted in a randomized block design with a 2×3×2 factorial arrangement, consisting of two hybrids (2B587 and 2B688; Dow AgroSciences), three genetic modification events (conventional, single event, and pyramidal event), and two crops seasons, with four replications. The conventional hybrids were not genetically modified materials. The single-event hybrids had the CRY1F and PAT genes (TC1507 and T25 events) from the non-pathogenic bacterium *Bacillus thurigiensis* var. aizawai, and the bacterium *Streptomyces viridochromogenes* strain Tu494; these genes express the proteins Cry1F and PAT, which have insecticidal effects on lepidopterans and generates tolerance to the ammonium glufosinate herbicide, respectively. Pyramidal hybrids had the genes Cry1F, Cry1A.105, and Cry2Ab2 (TC1507 and MON89034 events), which promote resistance to insects by producing proteins with insecticidal effect on lepidopterans; and the CP4 EPSPS and PAT genes (NK603 and T25 events), which promote tolerance to the glyphosate and ammonium glufosinate herbicides, respectively.

The 2B587 and 2B688 hybrids are single and triple type, with estimated growing degree units (GDU) of 815 and 860, respectively. The sowing density of the hybrids was 120 thousand seeds ha^{-1}. The plants were thinned at stage 3 (BBCH scale) to a population of 65,000 plants ha^{-1}. Weed control was carried out at thirty days after emergence by applying 1,500 g ha^{-1} of atrazine.

The plots consisted of four 5.0 m rows, spaced 0.8 m apart. The two central lines of the plots were used for the evaluations.

Plant height (PH), ear height (EH), and stem diameter (SD) were evaluated when the plants reached stage 10.5 (BBCH scale), considering the mean of ten plants randomly chosen in the plots. PH and EH were measured from the soil surface to the insertion point of the flag leaf and to the insertion point of the main ear, respectively. SD was measured at 5 cm above the main ear, using a digital caliper.

Grain yield (GY) of the plots was determined using manually harvested ears from plants at physiological maturation. The ears were threshed, and the grains weighed and sampled to determine their moisture, using a grain moisture meter (G-300; Gehaka, São Paulo, Brazil). Grain yield data were corrected to 13% moisture.

The data of the responses of the variables to the treatments were subjected to tests of normality and homogeneity of variance (BOX; COX, 1964), and to analysis of variance (ANOVA) using the F test at 5% probability. When the effects or interactions were significant, the means of the treatments were compared by the Tukey's test at 5% probability. All analyzes were performed using the SAS 9 (Statistical Analysis System) program.

Results and discussion

The crop season and hybrid factors were significant for PH and SD. The genetic modification event factor was significant for PH and EH. These interactions between crop season and hybrid and between hybrid and genetic modification event were significant for EH (TABLE 1). The 2B688 hybrid presented higher plant height, ear height, and stem diameter at the first reproductive stage of maize plants (TABLE 1). The results corroborate information presented by Embrapa (2016). These results were due to the different genetic bases of the hybrids (PENARIOL et al*.*, 2003; CARDOSO et al*.*, 2003).

The conventional hybrids achieved, in general, lower plant and ear height than single and pyramidal transgenic hybrids (TABLE 1). Modified plants for glyphosate tolerance may have overproduction of the EPSPS enzyme (GRUYS; SIKORSKI, 1999), especially in crops without application of the herbicide (YAMADA; CASTRO, 2007). The overproduction of the EPSPS enzyme increases the production of aromatic amino acids (tyrosine, phenylalanine, and tryptophan), which are compounds that directly affect plant height (SCHANBENBERGER et al*.*, 1999; WAGNER et al*.*, 2003; CEDERGREEN et al*.*, 2007; GODOY, 2007; CARBONARI et al*.*, 2007a; CARBONARI et al*.*, 2007b; VELINI et al*.*, 2008).

Similarly, modified plants with genes for insect-resistance, such as single-event hybrids, have overproduction of crystals called delta endotoxins or Cry proteins (AGAISSE; LERECLUS, 1995). Cry proteins are produced in the stationery and sporulation phases and accumulated in the mother cell, corresponding to 25% of the cell dry weight (AGAISSE; LERECLUS, 1995). Overproduction of Cry protein may also have affected the growth of the plants, increasing their heights. In addition, conventional plants were visually more harmed by pest insect attacks than resistant plants.

Table 1 – Means of plant height (PH), ear height (EH), and stem diameter (SD) of two maize hybrids (2B587 and 2B688) with different genetic modification events—conventional (CV), single event (SE), and pyramidal event (PE). Inconfidentes, Minas Gerais, Brazil.

		PH		EH		SD	
		2013/2014	2014/2015	2013/2014	2014/2015	2013/2014	2014/2015
		---------------------------- m ----------------------------				----------- mm -----------	
2B587	CV	2.27	2.25	1.22	1.31	19.22	16.66
2B587	SE	2.43	2.27	1.32	1.31	19.63	16.57
2B587	PE	2.36	2.34	1.24	1.30	20.61	17.02
Mean of the 2B587 hybrid		2.32B		1.28		18.29B	
2B688	CV	2.37	2.23	2.28	1.18	18.66	16.54
2B688	SE	2.52	2.38	1.36	1.30	18.62	16.59
2B688	PE	2.56	2.47	1.40	1.39	19.04	16.88
Mean of the 2B688 hybrid		2.42A		1.32		18.56A	
Mean of crop seasons		2.42a	2.32b	1.30	1.29	19.29a	16.71b
Means of events among hybrids and crop seasons							
	CV	2.28B		1.25		17.77	
	SE	2.40A		1.33		17.85	
	PE	2.43A		1.34		18.38	
ANOVA (Pr > F)							
Pr>F	Crop season (CS)	0.0002**		0.7117		<0.0001***	
	Hybrid (H)	0.0001**		0.0719		0.0391*	
	CS*H	0.2226		0.0044*		0.0998	
	Event (E)	<0.0001***		0.0008**		0.083	
	CS*E	0.1933		0.4762		0.8259	
	H*E	0.1037		0.0034**		0.7563	
	CS*H*E	0.4134		0.2326		0.7723	
CV %		5.4		7.2		16.5	

Means followed by different uppercase letters in the columns, or different lowercase letters in the rows are different by the Tukey's test (P <0.05).

Source: Prepared by the authors (2018).

The conventional 2B688 hybrid presented the shortest ear height, differing from its transgenic versions. The 2B587 hybrid presented a smaller ear height than the 2B688 when comparing their pyramidal-event versions (TABLE 2). Morphological changes in maize hybrids, such as plant and ear height, may affect cultural practices, such as harvesting, and the plant's response to stress conditions (SANGOI et al., 2002). These results indicate that transgenic maize hybrids are higher, and in some cases, have higher ear height than conventional ones. The increase in ear and plant height did not affect the stem diameter, which is an important characteristic to avoid tipping, especially in modern hybrids, which are sown at higher densities.

Table 2 – Means of ear height (EH) of two maize hybrids (2B587 and 2B688) with different genetic modification events - conventional (CV), single event (SE), and pyramidal event (PE). Inconfidentes, Minas Gerais, Brazil.

	Event		
Hybrid	CV	ES	EP
2B587	1.26 Aa	1.32Aa	1.27Ba
2B688	1.23 Ab	1.33 Aa	1.40 Aa
	Crop Season		
	2013/2014	2014/2015	
2B587	1.26 Ba	1.31 Aa	
2B688	1.35 Aa	1.29 Aa	

Means followed by different uppercase letters in the columns or different lowercase letters in the rows are different by the Tukey's test (P <0.05).
Source: Prepared by the authors (2018).

The genetically modified hybrids had higher mean yield than the conventional ones, with approximately 11,000 kg ha^{-1} (TABLE 3). Grain yield is the result of quantitative inheritance and is affected by many factors. Therefore, its correlation with a single genotypic trait is generally low. In addition, this difference may increase under favorable environmental conditions for the use of transgenic hybrids, which was probably the case in the present study.

The mean yield of the genetically modified hybrids was approximately 900 kg ha^{-1} (10%) higher than that of the conventional hybrids. These results are consistent with the range of losses caused by the maize armyworm (CARVALHO, 1970; CRUZ; TURPIN, 1983; WILLIAMS; DAVIS, 1990; CORTEZ; WAQUIL, 1997; CRUZ et al*.* 1999). In addition, the experimental area had been cultivated with maize for several consecutive crop seasons, Brazil's tropical climatic conditions allow this intensification of production (PATERNIANE, 2000), consequently, there were probably significant increases in pest insect populations (HILL, 1983; HOLLINGSWORTH, 2011; OMOTO et al*.* 2015).

Table 3 – Means of grain yield of two maize hybrids (2B587 and 2B688) with different genetic modification event – conventional (CV), single event (SE), and pyramidal event (PE). Inconfidentes, Minas Gerais, Brazil.

	Yield (kg ha^{-1})			
	Event	CV	SE	PE
2B587	2013/14	9.300	11.000	10.600
	2014/15	10.900	11.400	11.200
2B688	2013/14	10.300	10.500	10.700
	2014/15	9.500	10.600	11.400
Means of the events		10.000 b	10.900 a	11.000 a
	ANOVA (Pr > F)			
Pr>F	Crop Season (CS)		0.1653	
	Hybrid (H)		0.4162	
	CS*H		0.1248	
	Event (E)		0.0157*	
	CS*E		0.8349	
	H*E		0.6178	
	CS*H*E		0.2129	
	CV		10.7	

Means followed by different letters in the rows are different by the Tukey's Test (P <0.05).
Source: Prepared by the authors (2018).

Conclusion

Genetic modifications of maize plants used in transgenic hybrids affect their morphological and productive characteristics.

Transgenic hybrids present 5% higher plant height and 10% higher grain yield than conventional hybrids.

Interferência de eventos transgênicos nos atributos morfológicos e produtivos do milho

Resumo

O potencial de utilização e de benefícios de plantas geneticamente modificadas é enorme e dinâmico e, quanto mais conhecimento científico e técnico a ele for agregado, mais alternativas de manejo agrícola sustentáveis são disponibilizadas. Porém, pouco se conhece sobre os efeitos da resistência a herbicidas e sobre o ataque de insetos nas características agronômicas de plantas de milho. A presente pesquisa foi conduzida com o objetivo de investigar o efeito dos eventos transgênicos sobre as características morfológicas e produtivas de híbridos de milho. Para isso, foi realizado um experimento de campo em duas safras utilizando híbridos convencionais e transgênicos isogênicos. As variáveis analisadas foram: altura de planta, altura de espiga, espessura de colmo e produtividade de grãos. A introdução de diferentes biotecnologias modificou as características morfológicas e produtivas de plantas de milhos. Versões transgênicas apresentaram altura de planta e produtividade de grãos 5 e 10%, respectivamente, maior em relação às isolinhas convencionais.

Palavras-chave: Proteína Cry1Ab. Enzima PAT. Enzima EPSPS. Rendimento de grãos. Interação ambiente *versus* genótipo.

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