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Phenotypic variations between non-transgenic and transgenic maize hybrids

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ABSTRACT

The use of transgenic maize (*Zea mays* L.) hybrids (Bt, RR, Bt-RR) has simplified crop husbandry, mainly due to a more effective control of pests and weeds. The effects of transgenes insertion on phenotypic traits of maize hybrids are not fully documented, especially without the incidence of pests and weeds. The objectives of this work were (i) to establish phenotypic differences in terms of phenology, growth and yield among a non-transgenic maize hybrid and their transgenic versions (Bt hybrid, RR hybrid and Bt-RR hybrid) and among the transgenic versions of other genetic background and (ii) to analyze the impact of crowding stress on inter-plant variability of the different traits under study. Field experiments were conducted in Buenos Aires (34°36'S, 58°26'W), Argentina during 2008–2009 (Exp 1), 2009–2010 (Exp 2) and 2010–2011 (Exp 3). Genotypes were cultivated at contrasting plant densities (6 and 12 pl m⁻² in Exp 1 and Exp 2) and with contrasting inter-plant spaces within the row (Exp 3), irrigated, without nutrient limitations, and with chemical and mechanical controls of weed and animal pests. At both plant densities, phenotypic variability of several traits among versions within each group was recorded. For example, the RR version of DK747 group had the shortest thermal time to flowering. The stacked transgenic hybrids DK747MGRR and DK190MGRR, exhibited the highest ear growth rate (EGR_{CP}) during the critical period for kernel set. However, both genotypes had the lowest reproductive efficiency (i.e. kernel number per unit of EGR_{CP}), that counterbalanced their higher EGR_{CP}. Only the Bt-RR version of DK747 showed a higher inter-plant variability (CV) of EGR_{CP} at low mean EGR_{CP} values a symptom of the intolerance of this genotype to crowding stress. Consequently, the different versions of each group of hybrids had a similar kernel number per plant and grain yield. We conclude that the introduction of transgenes may alter in some way, the behavior of plants in different eco-physiological aspects in the absence of pests and weeds.

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1. Introduction

Maize crops in Argentina are mostly cultivated under rainfed conditions; therefore the main climatic constraint is the water supply around flowering (Hall et al., 1982); i.e. the most critical period for kernel set (Tollenaar et al., 1992; Andrade et al., 1999). So, in the temperate areas of the Pampas, maize crops are sown in early spring, and flowering occurs at the end of this season, before the seasonal summer drought. Another possibility to avoid this climatic stress is to sow maize crops at the end of the spring season (i.e. November–December) (Maddonni, 2012). However, the traditional non-transgenic maize hybrids cultivated at these late sowings are severely affected by biotic stress such as the attack of stem borer (*Diatraea saccharalis*) and armyworm (*Spodoptera frugiperda*) (Wiatrak et al., 2004). After the appearance of transgenic maize hybrids resistant to Lepidoptera, i.e. maize

capable of expressing the Cry1Ab insecticidal protein produced by the bacterium *Bacillus thuringiensis* (Bt hybrids; Williams et al., 1997), the late sowed maize crops have spread in Argentina with very good results.

Another agronomic practice with great popularity in the crop production systems in Argentina is the zero tillage. In this context, the use of transgenic maize hybrids resistant to the herbicide glyphosate (RR hybrids) has simplified the tasks of weed control and/or reduced the costs involved in this practice (Norsworthy and Frederick, 2005). Thus, the current portfolio of Argentine maize hybrids includes both non-transgenic and their near isogenic versions with single events or stacked events (tolerance to various Lepidoptera genera plus resistance to glyphosate; Bt-RR hybrids) for crop protection.

Several studies have been published since the release of Bt maize hybrids in different countries. Most of them were focused on the ecological impact of Bt-hybrids cultivation (e.g. Naranjo, 2009; Yu et al., 2011). Others studies have compared the performance of the non-transgenic maize hybrids and their Bt-versions to describe the beneficial effects of Bt technology in terms of biomass

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production and grain yield (Traore et al., 2000; Archer et al., 2001; Stanger and Lauer, 2006; Subedi and Ma, 2007; Coulter et al., 2010). Some of these studies have attributed the benefits of Bt technology to the lower stalk lodging or drop of the ears, i.e. higher harvest index (e.g. Stanger and Lauer, 2006), while other study have also reported a higher grain yield of the Bt hybrids than their non-transgenic versions, without incidence of Lepidoptera (Subedi and Ma, 2007). Additionally these authors detected a different phenology (e.g. time to female flowering) of the non-transgenic hybrids and their Bt versions, which was not associated with pest incidence (Ma and Subedi, 2005). Collectively, mentioned studies suggest that transgene introduction may alter plant physiology. Up to now, no information exists on the performance of stacked maize hybrids (i.e. Bt-RR hybrids) in comparison with the single transgenic hybrids and the non-transgenic versions, especially in the absence of the effect of biotic agents.

Under a population approach, maize grain yield is the sum of the individual performance of each plant that comprises it. Various studies (Edmeades and Daynard, 1979; Vega and Sadras, 2003; Maddonni and Otegui, 2006; Boomsma et al., 2009; Caviglia and Melchiori, 2011; Mayer et al., 2012) have documented a reduction in the average yield of plants and an increase in population variability of this trait when maize crops are exposed to abiotic stress (light, water and nutrients). This variability detected from early ontogenetic stages (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007), may be promoted by a different environment perceived by plants, by genetic variation among plants, or by both factors.

Environmental differences may be caused by subtle changes in initial conditions of plant growth related to an uneven seedling emergence (Pommel et al., 2001; Liu et al., 2004). Genetic differences among plants of a non-transgenic single-cross hybrid (i.e. individuals genetically identical) could be related to the degree of heterozygosity of the parent inbred lines, due to residual heterozygosity, genetic contamination during multiplication of the line, natural mutations or combination of these causes (Fleming et al., 1964). The existence of genetic variation within inbred lines has been quantified through the individual variability in phenotypic traits (Higgs and Russell, 1968) and by using molecular markers (Liu et al., 2003). The authors conclude that the detection of a small but significant genetic variation (4–5%) indicates that homozygosity across all loci cannot be assumed. In transgenic hybrids, the incorporation of the event is done by crossing a line source of the trans-gene and successive backcrosses to the original line. In these backcrosses, the linkage drag of the donor parent increases the likelihood of heterozygous loci in the converted line (Tanksley and Nelson, 1996). Therefore, the way of obtaining a transgenic hybrid could contribute to a genetic variability of various traits in culture conditions. Thus, there may be differences in some traits and/or in the inter-plant variability of these traits between a non-transgenic hybrid and its transgenic version. The different inter-plant variability could be expressed under environments with lower resources availability.

The objectives of this study were (i) to establish phenotypic differences in terms of phenology, growth and yield among a non-transgenic maize hybrids and their transgenic versions (Bt hybrid, RR hybrid and Bt-RR hybrid) and among the transgenic versions of other genetic background and (ii) to analyze the impact of crowding stress on inter-plant variability of the different traits under study.

2. Material and methods

2.1. Experimental design

Two field experiments were hand sown on October 30, 2008 (Exp 1) and October 30, 2009 (Exp 2) in the experimental field of

the Faculty of Agronomy of the University of Buenos Aires (34°35'S, 58°29'O) on a loam soil. For both experiments, maize was the previous crop. The top soil (0–20 cm layer) had an organic matter content of 27 g kg⁻¹, mean mineral P content of 17.3 mg kg⁻¹ and organic N of 1.5 g kg⁻¹. Treatments were evaluated using a split plot design arranged in a randomized complete block with three replicates. Plant population density was assigned to the main plots (D₆: 6 pl m⁻² and D₁₂: 12 pl m⁻²) and hybrids to the sub-plots (hereafter referred as plot). Each plot consisted of three rows 5 m long, spaced at 0.5 m. Plots were over-planted and then thinned to reach the desired plant density. Tested genotypes were commercial single-cross hybrids representative of two different genetic backgrounds. The DK747 group, composed by the non-transgenic DK747 and their transgenic versions: the Bt hybrid (DK747MG), the RR hybrid (DK747RR) and the Bt-RR hybrid (DK747MGRR). The DK190 group composed by the Bt hybrid (DK190MG), the RR hybrid (DK190RR) and the Bt-RR hybrid (DK190MGRR). Unfortunately, the non-transgenic version of the DK190 was not tested in this study because it was previously removed from the market.

In order to explore a wide range of inter-plant variability of the traits under study, a third field experiment (Exp 3) was sown in the field of the Faculty of Agronomy on October 28, 2010. Maize was also the previous crop. The same hybrids were arranged in plots of 15 m long and 2 m wide in a single repetition. Each plot comprised 4 rows spaced at 0.5 m. In each plot plant to plant distance was: 5 cm in the 1st row, 10 cm in the 2nd row, 20 cm in the 3rd row and 40 cm in the 4th row.

All experiments were sown from the same pool of seeds and conducted with supplemental irrigation (drip irrigation) and mechanical and chemical (atrazine 41 ha⁻¹) weed controls and chemical controls of pests (chlorpyrifos 11 ha⁻¹; cypermethrin 245 ml ha⁻¹). At the six-ligulated leaf stage (V₆; Ritchie et al., 1993), N fertilization (150 kg N ha⁻¹ as urea) was hand applied along the rows and immediately incorporated to the soil with a hoe.

2.2. Phenology and plant growth

In Exp 1 and Exp 2, 10 consecutive plants of equal size and similar ontogenetic stage were tagged early (V₃) in the middle row of each plot. On these tagged plants vegetative stages (V_n) were weekly recorded and male (anthesis, i.e. at least one anther releasing pollen) and female (at least a visible stigma out of the husk; R₁) flowering were registered to compute the interval between anthesis and silking dates (ASI) of each plant. In Exp 3, 40 plants of each hybrid were also identified in V₃. These plants were located in an area of 2 m², comprising ca. one lineal meter of each of the 4 rows of each plot (ca. 20 plants row⁻¹ in the 1st row, 10 plants row⁻¹ in the 2nd row; 5 plants row⁻¹ in the 3rd row and 3 plants row⁻¹ in the 4th row). In these plants phenological events were recorded as in Exp 1 and Exp 2.

To characterize the individual growth of the tagged plants along the cycle, a non-destructive method based on allometric models was used to estimate plant biomass from morphometric variables (Vega et al., 2001b; Maddonni and Otegui, 2004; Pagano and Maddonni, 2007; Rossini et al., 2011). For the construction of these models, in Exp 1 and Exp 2, 10–20 plants per hybrid were weekly sampled from V₃ to R₁ (when 50% of the plants in each plot had visible stigmata) and at 15 days of R₁ (i.e. R₂). In Exp 3, 11 plants per hybrid (5 plants from the 1st row, 3 plants from the 2nd row, 2 plants from the 3rd row and 1 plant from the 4th row) were sampled 7 days before R₁ and 30–40 plants per hybrid (ca. 20 plants from the 1st row, 10 plants from the 2nd row, 5 plants from the 3rd row and 2–3 plants from the 4th row) were sampled at R₁ and R₂ of each plant, i.e. the last two samplings were not conducted on a fixed date but in accordance with the phenology of each plant. For this objective, two sampling areas were delimited during the

pre-flowering period of each hybrid. In one area plants were harvested at R_1 and in the other area the silking date of each plant was registered and they were sampled 15 days after R_1 . In all sampled plants, measurements involved maximum and minimum diameter of the stem base and the length of the stem from soil surface to the insertion of the last fully expanded leaf. At R_1 and R_2 the maximum diameter of the apical ear was also measured. Sampled plants were then individually bagged and dried (60 °C) to constant weight to obtain the individual plant biomass (all samples) and ear shoot biomass (at R_1 and R_2).

From biomass data and allometric measurements, linear and bi-linear models were constructed to estimate total plant biomass from V_3 to R_1 and vegetative biomass (stem + leaves + tassel) at R_2 . For all hybrids, total biomass from V_3 to R_1 (Eq. (1) and Table 1) and vegetative biomass at R_2 (Eq. (4) and Table 1) was linearly related to the volume of a cylinder according to the average diameter of the stem and stem length ($\pi \times$ average radius of the base of the stem² × stem length). The only exception was the model of total biomass of hybrid DK190MG in Exp 3, in which the best fit of data (r^2) was obtained with a bi-linear model (Eqs. (2) and (3) and Table 1). Total biomass models had an intercept equal to zero to increase their sensitivity to low values of cylinder volume (i.e. early growth stages).

An exponential function was used to estimate ear shoot biomass from maximum ear diameter (Eq. (5) and Table 1).

$$\text{Total biomass (g)} = a \text{ volume} \quad (1)$$

$$\text{Total biomass (g)} = b \text{ vol, for volume} < c \quad (2)$$

$$\text{Total biomass (g)} = b c + d(\text{vol} - c), \text{ for volume} > c \quad (3)$$

$$\text{Vegetative biomass (g)} = e \text{ vol} + f \quad (4)$$

$$\text{Ear shoot biomass (g)} = g D^h \quad (5)$$

where a, b, c, d, e, f, g and h are parameters of the adjusted models, volume of the cylinder (in cm³) and D is the maximum diameter of the apical ear (in mm).

Morphometric measurements were also performed on tagged plants used for the records of phenology and growth, at the same time that plants were sampled to build the models. During the pre-flowering period, total plant biomass of each genotype was calculated from measurements of stem diameter and stem length using Eqs. (1)–(3). Ear shoot biomass at R_1 and R_2 was estimated from measurements of maximum ear diameter by means of Eq. (5). Total plant biomass at R_2 was calculated from estimated vegetative biomass (Eq. (4)) plus estimated ear shoot biomass (Eq. (5)). Finally, at physiological maturity all tagged plants were harvested and dried to measure total plant biomass, and grain yield per plant (GY). Kernel number per plant (KNP) was hand counted and kernel weight was estimated as the quotient between grain yield per plant and KNP.

2.3. Plant growth rate, biomass partitioning and kernel set

In all experiments, plant growth rate (in g d⁻¹) of each tagged plant was estimated from the slope of the linear regression fitted to plant biomass and time (in days) from sowing (Rossini et al., 2011) for the following periods: (i) the vegetative period, from V_3 to V_6 (PGR_V), (ii) the early reproductive period, from V_7 to V_{15} (PGR_{ER}) and (iii) the critical period, from R_1 -15 days (Exp 1 and Exp 2) or R_1 -7 days (Exp 3) to R_2 (PGR_{CP}). Ear growth rate (EGR_{CP}) was computed for the critical period (in g d⁻¹), and was based on (i) an ear biomass value of zero at 227 °Cd before R_1 (Otegui and Bonhomme, 1998) using the thermal time model with a base temperature 8 °C (Ritchie and NeSmith, 1991), (ii) the estimated ear biomass at R_1 , and (iii) the estimated ear biomass at R_2 .

Biomass partitioning to the ear was estimated through the ratio of (i) EGR_{CP} and PGR_{CP} (Pagano and Maddonni, 2007) and (ii) ear shoot biomass (cob + husk + grains) and plant biomass at R_6 . The harvest index of each plant (the ratio between grain yield and total plant biomass) was also computed.

Kernel number per plant of apical ear (KNP_{AE}) was related to PGR_{CP} and EGR_{CP} (Vega et al., 2001b) by a nonlinear model (Eqs. (6) and (7)).

$$\text{KNP}_{\text{AE}} = \frac{[i(\text{PGR}_{\text{CP}} - j)]}{[1 + k(\text{PGR}_{\text{CP}} - j)]}, \text{ for } j > \text{PGR}_{\text{CP}} \quad (6)$$

$$\text{KNP}_{\text{AE}} = \frac{[l(\text{EGR}_{\text{CP}} - m)]}{[1 + n(\text{EGR}_{\text{CP}} - m)]}, \text{ for } m > \text{EGR}_{\text{CP}} \quad (7)$$

where i and l are the initial slope, j and m are the threshold values of PGR_{CP} and EGR_{CP}, respectively to set a kernel (i.e. KNP_{AE} > 0) and k and n represent the curvilinear degree for high PGR_{CP} and EGR_{CP} values, respectively.

Reproductive efficiency (RE) of each plant was calculated from the ratio between KNP_{AE} and EGR_{CP} (Vega et al., 2001a).

2.4. Data analysis

The fit of the allometric models was performed with TBLCURVE (Jandel, 1992) and differences between years and genotypes in the adjusted model parameters were analyzed with confidence intervals at 95%.

In Exp 1 and 2, treatment effects (density and hybrid) and their interactions on measured and calculated variables through the years, were evaluated by analysis of variance (ANOVA). The ANOVA was performed with the experiment as a random variable and genotype and stand density as fix variables. Differences among genotypes were established using Fisher's protected least significant difference. Previously a test of equality of variance was performed. This data analysis was performed with Statistix 7.0 (STATISTIX, 2000).

Relationships among variables of the whole data set (Exp 1, Exp 2 and Exp 3 data) of each genotype were tested with correlation and regression analysis using TBLCURVE (Jandel, 1992).

For the data set of each group of hybrids, boundary lines were constructed for the relationship between KNP_{AE} and EGR_{CP} with the method of regression by quartiles of the program R (R Development Core Team, 2008) using the quartile 0.95. The inverse of this function would be indicative of the highest RE for each EGR_{CP} value. The root mean square error (RMSE, Eq. (8), Potter and Williams, 1994), estimated with actual RE values (KNP_{AE} EGR_{CP}⁻¹) and the highest RE values, was used to quantify the dispersion of data set of each genotype.

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^N (\text{RE}_{\text{Ai}} - \text{RE}_{\text{Hi}})^2}{N}} \quad (8)$$

where RE_{Ai} represents each actual RE, RE_{Hi} represents the highest RE for each EGR_{CP} value and N is the number of observations for each genotype.

In Exp 1 and Exp 2, interplant variability was characterized by the coefficient of variation (CV) of individual traits of each tagged plant plot⁻¹. In the case of anthesis and silking dates, a value of zero was assigned to the day before each event was first observed. Because negative and positive values can be obtained for the ASI and may cause mean values of zero that do not allow estimation of a CV, a correction was introduced to the whole data set. The most negative value was set to 0 and the rest modified accordingly. The CVs of all traits were analyzed by ANOVA after arcsine transformation when the data did not fulfill the assumption of normality. In Exp 3, for each hybrid the means and their corresponding CVs were

Table 1
Parameters of the allometric models used to estimate: (i) total plant biomass (g) during the pre-silking period (V_3 – R_1 for Exp 1 and Exp 2, V_{16} – R_1 for Exp 3), (ii) vegetative plant biomass (g) at 15 days after silking (R_2), and (iii) ear biomass (g) at silking (R_1) and at 15 days after silking (R_2). The coefficient of determination (r^2) and the number of data (n) used for model fitting are indicated.

Hybrid	Total plant biomass during the pre-silking period									
	Exp 1		Exp 2		Exp 3					
	<i>a</i>	r^2, n	<i>a</i>	r^2, n	<i>a</i>	r^2, n	<i>b</i>	<i>c</i>	<i>d</i>	r^2, n
DK747	0.156	0.93, 76	0.115	0.91, 73	0.1291	0.93, 43				
DK747MG	0.18	0.96, 76	0.119	0.93, 75	0.1253	0.86, 39				
DK747RR	0.144	0.94, 75	0.113	0.91, 74	0.1293	0.85, 47				
DK747MGRR	0.151	0.95, 73	0.143	0.94, 74	0.131	0.91, 40				
DK190MG	0.145	0.89, 75	0.159	0.95, 73			0.172	524.14	0.049	0.90, 44
DK190RR	0.174	0.89, 75	0.151	0.96, 73	0.129	0.80, 39				
DK190MGRR	0.164	0.92, 74	0.157	0.94, 73	0.134	0.81, 40				
Group	Vegetative plant biomass at R_2									
	Exp 1			Exp 2			Exp 3			
	<i>e</i>	<i>f</i>	r^2, n	<i>e</i>	<i>f</i>	r^2, n	<i>e</i>	<i>f</i>	r^2, n	
Group DK747	0.0831	71.024	0.71, 37	0.1094	43.95	0.72, 40	0.1026	17.646	0.9, 123	
Group DK190	0.1243	37.045	0.79, 30	0.1049	47.12	0.77, 30	0.1074	19.691	0.9, 85	
Hybrid	Ear biomass at R_1 and R_2									
	Exp 1 and Exp 2			Exp 3						
	<i>g</i>	<i>h</i>	r^2, n	<i>g</i>	<i>h</i>	r^2, n				
DK747	0.0022	25.663	0.94, 37	0.0003	30.086	0.95, 67				
DK747MG	0.001	27.559	0.97, 38	0.0003	29.773	0.97, 59				
DK747RR	0.0008	28.556	0.96, 37	0.0005	28.542	0.94, 65				
DK747MGRR	0.0006	29.495	0.96, 37	0.0003	30.516	0.97, 58				
DK190MG	0.0009	27.875	0.95, 37	0.0005	28.738	0.97, 67				
DK190RR	0.0002	31.331	0.97, 35	0.0002	3.148	0.96, 51				
DK190MGRR	0.0004	30.301	0.97, 37	0.0003	29.979	0.97, 56				

calculated from individual values of each trait of plants of the 1st row, 2nd row and 3rd + 4th rows.

In order to determine the effect of crowding stress on the response of the CV to mean values of tested traits (PGR_{ER} , PGR_{CP} , EGR_{CP} , KNP_{AE} , RE and GYP), the CVs were associated with the corresponding mean value of each plot (Exp 1 and Exp 2) or row/s (Exp 3). A negative exponential function (Tollenaar and Wu, 1999) was fitted (GraphPadPrism 5.00 for Windows) to the CVs and mean values of all traits (Eq. (9)). Parameters of fitted functions were compared by *F*-tests among genotypes of each group.

$$CV = (o - p)e^{(-qx)} + p \quad (9)$$

where x is the mean value of the trait, o is the CV value at x close to zero, p is the minimum CV value (plateau), and q is a coefficient that characterizes the degree of CV reduction as mean trait value increases.

3. Results

3.1. Plant growth rate

In both experiments (Exp 1 and Exp 2), increasing plant density reduced PGR_{CP} ($0.0001 < P < 0.01$, Table 2). Only during Exp 2 ($P < 0.05$ for experiment $\times$ plant density), this effect was detected from earlier stages of maize phenology (i.e. PGR_V) and sustained throughout the cycle (i.e. PGR_{ER} and PGR_{CP}).

Differences in plant growth rate among versions of each genetic background were detected after V_6 , as PGR_V only differed ($P < 0.1$) between groups of hybrids (ca. 0.50 and 0.57 g d⁻¹ for DK747 versions and DK190 versions, respectively). Significant ($P < 0.05$) experiment $\times$ hybrid $\times$ plant density interactions on PGR_{ER} and PGR_{CP} were detected. Thus in both experiments, DK747MGRR at D_6 and DK747MG at D_{12} exhibited the largest PGR_{ER} of DK747

group. Contrarily, within the group of DK190, significant differences ($P < 0.05$) among genotypes on PGR_{ER} were only detected at D_6 . At this density, DK190RR and DK190MGRR in Exp 1 and DK190MG in Exp 2 exhibited the largest PGR_{ER} .

In Exp 1, the largest PGR_{CP} were attained by DK747RR and DK747MGRR at both densities, and by DK190MG at D_6 and DK190MGRR at D_{12} . By contrast during Exp 2, no differences were detected in the PGR_{CP} among the different versions within each group of hybrids.

3.2. Dynamics of male (anthesis) and female (silking) flowerings and ASI

In Exp 1 and Exp 2, thermal time to anthesis and silking of all genotypes increased in response to increased plant density ($P < 0.001$, Table 2). Hybrids of DK747 group had a shorter thermal time to anthesis and silking than those of DK190, and DK747RR exhibited the shortest thermal times to both floral events at both densities ($P < 0.01$, Table 2). By contrast, only DK190RR in Exp 2 at D_{12} had a shorter thermal time to anthesis than the other transgenic versions of DK190 group.

Differences between groups of hybrids in the thermal time to anthesis were partially related to total leaf number ($P < 0.001$). Hybrids of DK190 group had a higher total leaf number (19–20 leaves) than those of DK747 group (18–19 leaves). This trait however, did not explain the shortest thermal time to anthesis of DK747RR and DK190RR.

As increased plant density promoted a higher delayed in thermal time to silking than to anthesis, the ASI at D_{12} was longer (ca. 1 days for hybrids of DK747 group and ca. 1.7 days for hybrids of DK190 group) than at D_6 ($P < 0.01$, Table 2). A significant hybrid $\times$ experiment $\times$ plant density interaction was detected for ASIs ($P < 0.05$, Table 2). In Exp 1 at D_{12} DK747 had the largest ASI

Table 2

Plant growth rate during the vegetative period (PGR_V), the early reproductive period (PGR_{ER}) and the critical period (PGR_{CP}), ear growth rate during the critical period (EGR_{CP}), biomass partitioning to the ear during the critical period (BPE_{CP}), biomass partitioning to the ear at physiological maturity (BE_{BF}^{-1}), harvest index (HI), grain yield per plant (GYP), kernel number per plant (KNP), kernel weight, reproductive efficiency (RE), thermal time to anthesis (TT anthesis), thermal time to silking (TT silking) and anthesis–silking interval (ASI) of hybrid (H) DK747 and their transgenic versions, and the transgenic versions of DK190, cultivated at two plant densities (D). Values are the means of Exp 1 and Exp 2 (E).

Hybrid	Density ($pl\ m^{-2}$)	PGR_V ($g\ d^{-1}$)	PGR_{ER} ($g\ d^{-1}$)	PGR_{CP} ($g\ d^{-1}$)	EGR_{CP} ($g\ d^{-1}$)	BPE_{CP}	BE_{BF}^{-1}	HI	GYP ($g\ pl^{-1}$)	KNP	Kernel weight (mg)	RE ($k\ d\ g^{-1}$)	TT anthesis ($^{\circ}C\ d$)	TT silking ($^{\circ}C\ d$)	ASI (d)
747	6	0.59	4.44	7.32	2.26	0.31	0.65	0.54	204.88	693.73	296.01	297.45	918.76	931.23	0.67
747MG	6	0.63	4.50	6.91	2.05	0.30	0.64	0.53	199.78	653.75	306.03	336.53	933.20	943.57	0.57
747RR	6	0.59	4.21	7.59	2.42	0.32	0.65	0.54	198.69	668.71	297.05	282.95	914.23	925.43	0.64
747MGRR	6	0.68	5.10	7.44	2.56	0.35	0.64	0.53	202.24	653.57	310.71	256.78	923.91	933.18	0.52
190MG	6	0.63	4.79	6.65	2.32	0.36	0.63	0.53	199.79	760.1	260.45	287.73	955.71	965.22	0.57
190RR	6	0.66	5.10	6.13	1.94	0.33	0.65	0.54	193.39	782.47	251.52	261.37	949.79	961.47	0.67
190MGRR	6	0.73	5.10	6.58	2.57	0.40	0.65	0.53	186.79	776.57	244.01	248.99	952.41	957.17	0.32
747	12	0.36	2.36	4.51	1.59	0.35	0.61	0.51	113.31	451.08	252.65	317.83	967.34	1001.80	2.05
747MG	12	0.45	2.85	4.57	1.45	0.32	0.61	0.51	114.02	465.35	245.25	362.28	969.56	1000.60	1.95
747RR	12	0.38	2.42	4.76	1.70	0.36	0.60	0.58	120.02	461.40	228.32	285.50	950.92	975.57	1.43
747MGRR	12	0.36	2.64	4.83	1.90	0.40	0.62	0.52	117.16	455.85	257.03	244.38	968.71	990.80	1.33
190MG	12	0.37	2.84	3.92	1.63	0.41	0.59	0.50	100.78	438.97	232.08	301.81	1001.30	1032.80	2.05
190RR	12	0.43	2.81	3.79	1.48	0.39	0.61	0.52	108.53	454.02	240.13	262.55	988.06	1018.30	1.87
190MGRR	12	0.62	2.77	4.41	1.97	0.46	0.61	0.52	107.64	458.05	235.28	260.86	986.56	1011.40	1.67
<i>Significance level of main effects and the interactions</i>															
E		*					**	*	*	*		**			***
D	**			***	***	**	**		***	***	**		**	***	**
E × D	*	*										†			
H	†		***	***	***	***	†			**	***	***	***	***	*
H × E		**	***			*									
H × D										***	***				
H × E × D		*	*										†		*

† Significance level at $P < 0.1$.

* Significance level at $P < 0.05$.

** Significance level at $P < 0.01$.

*** Significance level at $P < 0.001$.

(ca. 3 d) and DK747RR and DK747MGRR the shortest ASIs (ca. 1.7 d) within the DK747 group. By contrast, in Exp 1 at D₁₂ and in Exp 2 at D₆ DK190MGRR had a shorter ASI (ca. 2.13 and 0.06 d, respectively) than DK190MG (2.80 and 0.43 d, respectively) but similar than that of DK190RR (ca. 2.56 and 0.26 d, respectively).

3.3. Biomass partitioning and ear growth rate

In Exp 1 and Exp 2 increased plant density determined a slight increase ($P < 0.01$, Table 2) in biomass partitioning to the ear during the critical period (BPE_{CP}) (ca. 0.34–0.37 at D₆ and D₁₂, respectively), but a decrease ($P < 0.001$, Table 2) of EGR_{CP} (ca. 2.3 and 1.6 g d⁻¹ at D₆ and D₁₂, respectively). At both densities, DK747MGRR (Exp 1) and DK190MGRR (Exp 1 and Exp 2) had the highest values of BPE_{CP} ($P < 0.05$ for hybrid $\times$ experiment, Table 2). In both experiments and densities, DK747MGRR, DK747RR and DK190MGRR had the highest EGR_{CP} ($P < 0.001$, Table 2). In Exp 3, plants of DK747MGRR with PGR_{CP} < 4 g d⁻¹ exhibited a sharp decline of BPE_{CP} (Fig. 1b).

At R₆ the proportion of total biomass allocated in the ear (BE BT⁻¹) (Table 2) differed between experiments (ca. 0.65 and 0.61 in Exp 1 and Exp 2, respectively, $P < 0.01$) and densities (ca. 0.64 and 0.60 at D₆ and D₁₂, respectively, $P < 0.01$), and DK190MG at both plant densities exhibited a slightly lower ($P < 0.1$) BE BT⁻¹ than the other transgenic versions of DK190 (Table 2).

The proportion of total biomass allocated in the kernels (i.e. harvest index) also differed among experiments (ca. 0.54 and 0.51 in Exp 1 and Exp 2, respectively, $P < 0.05$, Table 2), but was similar among genotypes and plant population densities.

3.4. Grain yield per plant, grain yield components and relationships between kernel number per plant, plant growth rate and ear growth rate around silking

Both grain yield per plant (ca. 161.92 and 147.65 g pl⁻¹ in Exp 1 and Exp 2, respectively) and KNP (ca. 602 and 565 kernels in Exp 1 and Exp 2, respectively) were higher ($P < 0.05$) in Exp 1 than in Exp 2 (Table 2). However, in both experiments, the increased plant density reduced grain yield per plant ($P < 0.001$), KNP ($P < 0.001$) and kernel weight ($P < 0.01$) (Table 2). Hybrids of DK190 group had a higher KNP than those of DK747 group only at D₆ ($P < 0.001$, for hybrid $\times$ plant density, Table 2), but a similar grain yield per plant due to a lighter kernel weight ($P < 0.001$ for hybrid $\times$ plant density, Table 2). Within each group of hybrids, grain yield per plant did not differ among versions because of their similar KNP and kernel weight (Table 2).

Only at the lowest plant density, all hybrids showed changes in the RE between experiments (ca. 255 and 309 kernels d g⁻¹ d in Exp 1 and Exp 2, respectively), whereas at the highest density hybrids reached a similar RE (ca. 290 kernels d g⁻¹) ($P < 0.1$ for experiment $\times$ plant density, Table 2). The Bt–RR versions of both groups of hybrids had the lowest RE at D₆ and D₁₂ ($P < 0.001$, Table 2). Note that the range of EGR_{CP} explored in these experiments did not include extremely low values of this trait because of the conditions of crop management (irrigation, fertilization, and D₁₂ as the highest plant density). For this reason, the response of KNP_{AE} to EGR_{CP} of the different versions of both groups of hybrids was analyzed in a wide range of EGR_{CP} (Exp 3) specifically covering lower values of this trait. For each hybrid, data set of individual plants of Exp 3 was pooled together with those of the other experiments. Under this analysis, DK747MGRR presented the steepest decline of KNP_{AE} at low PGR_{CP} ($i = 336$ and 241 kernels d g⁻¹ for DK747MGRR and the other versions, respectively) and the highest sterility threshold ($j = 2$ and 0.43 g d⁻¹ for DK747MGRR and the other versions, respectively) within the group of DK747 (Fig. 1a). Similarly, when the response of KNP_{AE} to PGR_{CP} was analyzed for

hybrids of DK190 group, the DK190RR had the greatest decline of KNP_{AE} at low PGR_{CP} ($i = 491$ and 283 kernels d g⁻¹ for DK190RR and the other versions, respectively) and the highest threshold of sterility ($j = 1.4$ and 0.14 g d⁻¹ for DK190RR and the other versions, respectively) (Fig. 1d).

To reduce the effect of genotypic differences in biomass partitioning to the ear during the critical period (Fig. 1b and e), the response of KNP_{AE} to EGR_{CP} was analyzed for the same set of data from three experiments (Fig. 1c and f). Parameters of fitted functions mainly differed among versions within each group. Within the group DK747, the DK747MGRR had the lowest setting of kernels at low EGR_{CP} ($l = 410$ and 688 kernels d g⁻¹ for DK747MGRR and the other versions, respectively) but also the lowest degree of curvilinearity ($n = 0.26$ and 0.63 kernels d g⁻¹ for DK747MGRR and the other versions, respectively) at high EGR_{CP}. Within the group DK190, the DK190RR also had the lowest setting of kernels at low EGR_{CP} ($l = 510$ and 720 kernels d g⁻¹ for DK190RR and the other versions, respectively) but an intermediate degree of curvilinearity ($n = 0.68$ kernels d g⁻¹) at high EGR_{CP}. Consequently, at low EGR_{CP} hybrids DK747MGRR and DK190RR showed the lowest RE values.

The analysis of the RE for the entire range of EGR_{CP} showed high inter-plant variability of the former at low EGR_{CP} and a reduced variability at high EGR_{CP}. In the group of hybrids DK747 (Fig. 1c insets), the largest mean deviations (RMSE) to the maximum RE (i.e. border line) corresponded to DK747MGRR (ca. 380 kernels d g⁻¹), followed by DK747RR (ca. 293 kernels d g⁻¹), DK747MG (ca. 279 kernels d g⁻¹) and DK747 (ca. 264 kernels d g⁻¹). Analyzing the RMSE for different EGR_{CP} intervals (interval 1 = EGR_{CP} < 1 g d⁻¹, interval 2 = 1 g d⁻¹ $< \text{EGR}_{\text{CP}} < 2$ g d⁻¹, interval 3 = 2 g d⁻¹ $< \text{EGR}_{\text{CP}} < 3$ g d⁻¹, EGR_{CP} interval 4 = EGR_{CP} > 3 g d⁻¹), the largest RMSE values were obtained in the interval 1 with the greatest deviations of DK747MGRR and the lowest ones of DK747. In the intervals 2 and 3 this trend was supported but differences among hybrids were reduced. Finally, for the interval 4 all versions had a similar RMSE value.

The same analysis was performed for the transgenic hybrids of DK190 (Fig. 1f insets). While the differences among versions were lower than those of DK747, DK190MG had the highest RMSE value (ca. 263 kernels d g⁻¹), followed by DK190MGRR (ca. 238 kernels d g⁻¹), and DK190RR (ca. 237 kernels d g⁻¹). The major differences in RMSE between versions were observed in the interval 1 and 4, with the highest RMSE of DK190MG in the interval 1 but of DK190MGRR in the interval 4.

3.5. Interplant variability

The higher CV values of most traits studied (PGR_V, PGR_{ER}, PGR_{CP}, EGR_{CP}, KNP, RE, GYP) were obtained at D₁₂ ($0.01 < P < 0.1$) while the CVs of the other traits had the same trend, but differences between densities were not significant (data not shown). Some traits showed greater variability ($0.01 < P < 0.1$) in Exp 1 (CV 0.17, 0.18, 0.16 and 0.21 for EGR_{CP}, BP_{PC}, RE and GYP, respectively) than in Exp 2 (CV 0.14, 0.17, 0.14 and 0.15 for EGR_{CP}, BP_{CP}, RE and GYP, respectively).

In Exp 1, the non-transgenic DK747 had the highest CV of PGR_V (0.29) in the group of DK747 (ca. 0.22 for the other versions), whereas in Exp 2, DK747MGRR reached the highest value of this trait (CV = 0.35 and 0.24 for DK747MGRR and the other versions, respectively). Experiment $\times$ hybrid; $P < 0.1$). In both experiments, DK747MGRR at D₁₂ presented a higher CV of PGR_{CP} (ca. 0.25) than DK747MG (ca. 0.19; hybrid $\times$ plant density; $P < 0.1$). However, at this density the non-transgenic DK747 had the highest CV of KNP (0.24 and ca. 0.16 for DK747 and other transgenic versions, respectively). Hybrid $\times$ plant density; $P < 0.001$). The transgenic hybrids of DK190 did not differ in the CVs of PGR_V (ca. 0.30), PGR_{ER} (ca. 0.21), PGR_{CP} (ca. 0.17), and KNP (ca. 0.17). Hybrid DK190MG, however,

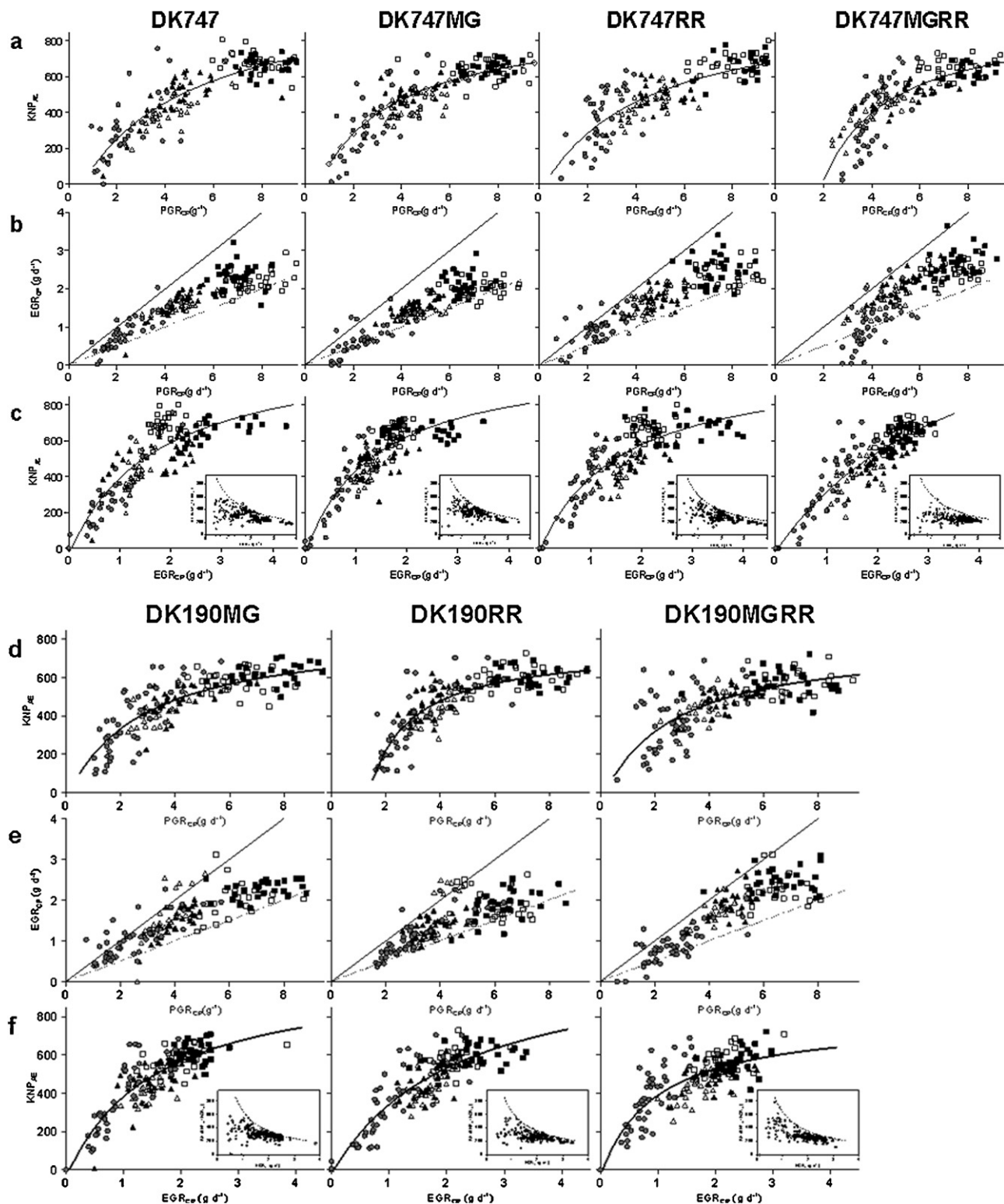


Fig. 1. Relationships between (i) kernel number per plant of the apical ear (KNP_{AE}) and plant growth rate during the critical period (PGR_{CP}) (a, d), (ii) ear growth rate during the critical period (EGR_{CP}) and PGR_{CP} (b, e) and (iii) KNP_{AE} and EGR_{CP} (c, f) of the non-transgenic DK747 and its transgenic versions (a–c) and the transgenic versions of DK190 (d–f). Symbols: squares (6 pl m⁻²); triangles (12 pl m⁻²); full symbols (Exp 1); empty symbols (Exp 2) and gray circles (Exp 3). The lines in (b) and (e) represent the 0.50 (solid) and 0.25 (dotted) ratios between EGR_{CP} and PGR_{CP} (i.e. biomass partitioning to the ear). The insets (c, f) show the relationships between the reproductive efficiencies (RE) and EGR_{CP} and dotted lines represent the maximum values of RE. For DK747 in (a): $i=224.6$, $j=0.532$, $k=0.207$ ($r^2=0.75$, $n=160$); in (c): $l=635.4$, $m=0.059$, $n=0.565$ ($r^2=0.78$, $n=160$). For DK747MG in (a): $i=276.8$, $j=0.538$, $k=0.298$ ($r^2=0.69$, $n=166$); in (c): $l=760.1$, $m=0.044$, $n=0.713$ ($r^2=0.81$, $n=166$). For DK747RR in (a): $i=223.1$, $j=0.2189$, $k=0.222$ ($r^2=0.71$, $n=164$); in (c): $l=647.4$, $m=0.029$, $n=0.618$ ($r^2=0.79$, $n=164$). For DK747MGRR in (a): $i=336.3$, $j=1.923$, $k=0.362$ ($r^2=0.69$, $n=168$); in (c): $l=410.5$, $m=0.049$, $n=0.259$ ($r^2=0.82$, $n=168$). For DK190MG in (d): $i=290.4$, $j=0.113$, $k=0.344$ ($r^2=0.66$, $n=165$); in (f): $l=643.8$, $m=0.066$, $n=0.618$ ($r^2=0.75$, $n=165$). For DK190RR in (d): $i=491.2$, $j=1.358$, $k=0.648$ ($r^2=0.71$, $n=165$); in (f): $l=509.9$, $m=0.044$, $n=0.449$ ($r^2=0.78$, $n=165$). For DK190MGRR in (d): $i=288.6$, $j=0.170$, $k=0.362$ ($r^2=0.65$, $n=171$); in (f): $l=793.5$, $m=0.042$, $n=0.996$ ($r^2=0.70$, $n=170$).

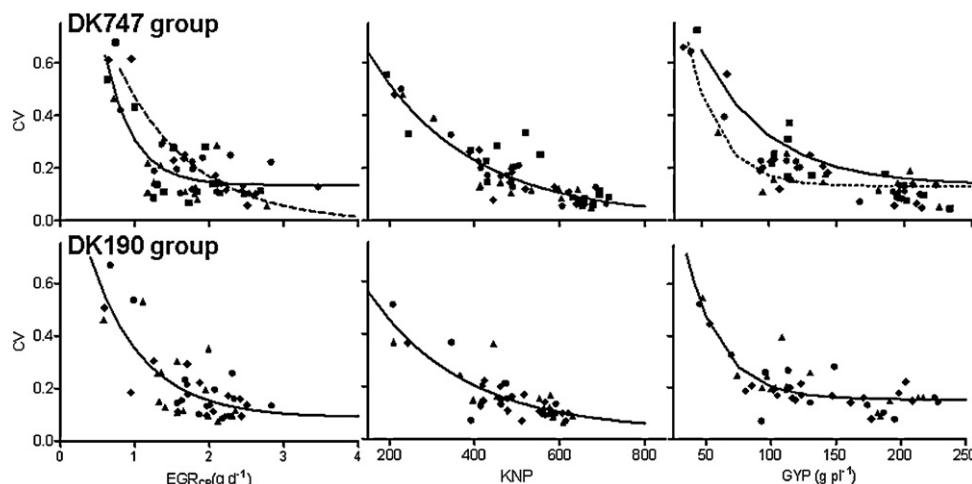


Fig. 2. Relationships between the coefficient of variation (CV) of EGR_{cp} (a, d), kernel number per plant (KNP) (b, e) and grain yield per plant (GYP) (c and f) and their respective means values of hybrids DK747 group (a–c) and DK190 group (d–f). Symbols: squares (DK747); triangles (DK747MG and DK190MG); circles (DK747RR and DK190RR); diamonds (DK747MGRR and DK190MGRR). Lines represent then fitted functions. In (a) solid line for DK747, DK747MG and DK747RR ($\sigma = 2.55$, $p = 0.14$, $q = 2.66$, $r^2 = 0.79$, $n = 45$), and dashed line for DK747MGRR ($\sigma = 1.28$, $p = -0.01$, $q = 0.99$, $r^2 = 0.92$, $n = 15$). In (b) solid line for all genotypes of DK747 group ($\sigma = 1.19$, $p = 0.01$, $q = 0.004$, $r^2 = 0.83$, $n = 60$). In (c) solid line for DK747, DK747RR and DK747MGRR ($\sigma = 1.47$, $p = 0.07$, $q = 0.02$, $r^2 = 0.90$, $n = 45$), and dotted line for DK747MG ($\sigma = 3.24$, $p = 0.13$, $q = 0.04$, $r^2 = 0.93$, $n = 15$). In (d) solid line for all genotypes of DK190 group ($\sigma = 1.16$, $p = 0.09$, $q = 1.41$, $r^2 = 0.61$, $n = 45$). In (e) solid line for all genotypes of DK190 group ($\sigma = 1.06$, $p = 0.03$, $q = 0.004$, $r^2 = 0.70$, $n = 45$). In (f) solid line for all genotypes of DK190 group ($\sigma = 2.24$, $p = 0.15$, $q = 0.04$, $r^2 = 0.71$, $n = 45$).

exhibited the highest CVs of BE BT⁻¹ at R₆ (0.14 and ca. 0.05 for DK190MG and the other versions, respectively).

The analysis of the response of interplant variability of the different traits to changes in the mean values, especially promoted by crowding stress, did not reveal a tendency for PGR_{ER} and RE (data not shown). Conversely, the CVs of the PGR_{CP}, EGR_{CP}, KNP and GYP significantly increased in response to the decrease of the mean values of these traits (Fig. 2; $0.59 < r^2 < 0.95$). A single negative exponential function was fitted to the CVs of PGR_{CP} and the mean values of all genotypes of each group (data not shown).

For the explored range of EGR_{CP}, all hybrids of DK747 group significantly increased the inter-plant variability (i.e. high CVs) of this trait as mean the values decreased (Fig. 2a; $r^2 \geq 0.74$). The DK747MGRR attained higher CVs for EGR_{CP} < 2 g d⁻¹ ($q = 0.99$ for DK747MGRR vs $q = 2.66$ for DK747, DK747MG and DK747RR) and lower CVs for EGR_{CP} > 2 g d⁻¹ ($p = -0.01$ for DK747MGRR vs $p = 0.13$ for DK747, DK747MG and DK747RR). For the transgenic versions of DK190, changes in the CVs of the EGR_{CP} were associated with the mean values of this trait and a single negative exponential function was fitted to the whole data set (Fig. 2d; $r^2 = 0.61$).

Changes in the CVs of KNP in response to the mean values of this trait were similar among different versions of DK747 and among versions of DK190. Both relationships were described by negative exponential functions (Fig. 2b; $r^2 = 0.83$; Fig. 2e; $r^2 = 0.70$).

Finally, the relationships between CVs of GYP and the mean values of this trait differed among versions of DK747 group (Fig. 2c). The DK747MG showed the lowest CVs but a sharply increase of this trait at low GYPs ($q = 0.04$ for the DK747MG vs $q = 0.02$ for DK747, DK747RR and DK747MGRR). On the contrary, hybrids of DK190 exhibited a similar response of the CVs of GYP to mean GYP values (Fig. 2f).

4. Discussion

In this study, we have analyzed several traits related to growth and development in a non-transgenic maize hybrid (DK747) and its transgenic versions (DK747MG, DK747RR and DK747MGRR) as well as in transgenic versions of another hybrid (DK190MG, DK190RR and DK190MGRR). Genotypes were cultivated at contrasting plant densities to analyze the responses of the traits to

crowding stress, the inter-plant variability of these traits and the stability in the mechanisms responsible for kernel setting (biomass partitioning to reproductive structures around the critical period and reproductive efficiency). For some of the evaluated traits the existence of phenotypic variability between groups of hybrids and among versions within each group was demonstrated. Some of these differences were independent of plant density and/or the environment of each experiment (i.e. constitutive responses) while others were detected only under some conditions (i.e. adaptive responses).

Differences in the growth and development among non-transgenic maize hybrids are expected responses (Otegui and Melon, 1997; Maddonni et al., 1999; Pagano and Maddonni, 2007; Pagano et al., 2007). Differences in mentioned traits between the transgenic versions and the non-transgenic version of a hybrid and among the transgenic versions of the same hybrid, may suggest a possible novel role of the introduced genes on the physiology and the phenotype of the plants. In both experiments the Bt version of DK747 (DK747MG) at the high plant density exhibited the highest PGR_{ER} but a similar inter-plant variability for this trait than the other versions of DK747 group. By contrast at the lowest density, the DK747 version with stacked transgenic events (DK747MGRR) exhibited the greatest PGR_{ER}. Hence, our results reveal the high early tolerance to crowding stress of DK747MG (i.e. high PGR_{ER} at high plant density) and the high growth of DK747MGRR under potential conditions (i.e. high PGR_{ER} at low plant density).

While there are evidences of genotypic differences in the early plant growth among non-transgenic maize hybrids (Pagano and Maddonni, 2007), no information exists in this response between the transgenic versions and the non-transgenic versions of the same hybrid. Physiological bases of plant growth, i.e. the efficiency of radiation interception and radiation use efficiency (Boote and Loomis, 1991), were not analyzed in this work. The first efficiency is dependent on plant leaf area and spatial leaf area distribution (Maddonni et al., 2001), while the second one is species specific (Kiniry et al., 1989), but may be altered by the content of foliar N (Sinclair and Horie, 1989). Considering that incident solar radiation during the early reproductive period was similar for all genotypes, variations in PGR_{ER} would have been caused by differences in the efficiency of radiation interception and/or radiation use efficiency.

In a recent study, at the beginning of the early reproductive period the Bt maize hybrid presented a similar leaf N concentration than the non-transgenic counterpart, but a greater vegetative growth (Subedi and Ma, 2007). Consequently, changes in the efficiency of radiation interception could explain the phenotypic variations detected in the early growth of plants between versions of DK747 group. These differences could be originated by epistatic effects (Falconer, 1981) or pleiotropic effects (Ge et al., 2004) related to the site of the genome where the transgene was inserted (Feldmann et al., 1989).

Despite a vigorous PGR_{ER} could increase the competitiveness of maize plants with other biotic components of the agro-ecosystem (e.g. weeds; Rajcan and Swanton, 2001), this high growth should be reflected in the underlying mechanisms of the reproductive success of plants (i.e. high EGR_{CP} and RE). Interestingly, not all genotypes with high PGR_{ER} (DK747MG at high density and DK747MGRR at low density) had the highest values of EGR_{CP} . Thus, both stacked transgenic hybrids (DK747MGRR and DK190MGRR) presented the highest EGR_{CP} at both plant densities (i.e. constitutive response) by their high biomass partitioning to the ear. Genotypic differences in biomass partitioning to the ear were documented between maize lines and hybrids (Echarte and Tollenaar, 2006) and among non-transgenic maize hybrids (Echarte et al., 2004; Pagano et al., 2007; D'Andrea et al., 2008) but this information have never been documented for different versions of the same hybrid. In maize, apical dominance exerted by the panicle regulates the proportion of biomass that is allocated in the ear during the critical period (Edmeades and Daynard, 1979). Various hormones are involved in the apical dominance (Thimann and Skoog, 1934; Eliasson, 1975; Blake et al., 1983; Russell and Thimann, 1988; Pilate et al., 1989). Differences in the synthesis, accumulation (Cline, 1994) or sensitivity to hormones that control a process (Trewavas, 1981; Cline, 1994) may affect its physiological responses. The introduction of a transgene into the endogenous genome of a plant could affect either (i) the expression of genes related to hormone synthesis and thereby modifies its final content or (ii) the genes related to the reception of hormonal signals changing the sensitivity to them. In rice, Wang et al. (2012) found variability in the content of certain growth promoting hormones (IAA, ZT and GA 3) between Bt genotypes (lines and hybrids) and their non-transgenic counterparts. These hormones may play a role in the apical dominance and biomass partitioning to reproductive organs. Moreover, previous studies in soybean (Elmore et al., 2001; de Vries and Fehr, 2011) have shown that RR lines were taller than non-transgenic lines, reflecting differences in the apical dominance among tested lines.

The existence of phenotypic variability in biomass partitioning to the ear during the critical period did not allow fitting a single model to predict KNP from the PGR_{CP} (Andrade et al., 2002) (Fig. 1a and d). Moreover, the relationship between KNP and EGR_{CP} was not the same for all genotypes (Fig. 1c and f) due to differences in the RE among versions. Differences in the KNP- EGR_{CP} relationship between new and old maize hybrids were previously reported (Echarte et al., 2004), but has never been reported among non-transgenic and transgenic versions of a hybrid. In our study, the DK747MGRR and DK190MGRR exhibited the lowest mean values of RE at both plant densities (i.e. constitutive response). When the RE was analyzed on a plant basis, the lower values were recorded for plants of DK747MGRR at low EGR_{CP} and for plants of DK190MGRR at high EGR_{CP} . Previous studies have shown that kernel abortion is the origin of the lower RE of the most suppressed plants of a stand (i.e. plants with low EGR_{CP}), and this via of kernel loss is determined by the great asynchrony of silks emergence (Otegui et al., 1995; Pagano et al., 2007; Rattalino Edreira et al., 2011). The lower RE of DK747MGRR plants at low EGR_{CP} was possibly controlled by this process. Plants of DK190MGRR also had low RE but at high

EGR_{CP} . Specific studies of transgenes effect on floral development will elucidate these genotypic differences.

The effect of Bt-transgene on time to flowering has been previously reported (Ma and Subedi, 2005). In the mentioned work Bt-maize genotypes exhibited a longer time to silking than the non-transgenic counterparts. In the present work, at the lowest plant density the Bt-version of DK747 exhibited a longer time to silking than the non-transgenic version, but this difference was not statistically significant. Interestingly, the DK747RR had the shortest time to flowering under all tested conditions (i.e. constitutive response). This is a novel result of transgene effects on maize development. This genotype did not have fewer leaves and/or a higher leaf emergence rate (i.e. phyllochrone, data not shown) than the other versions of its group to determine its early flowering (Tollenaar et al., 1979). Changes in the synthesis of hormones could also be modulating this reproductive trait. On the other hand, the Bt-RR version of DK747 did not change the time to flowering, suggesting a possible interaction of both transgenes on floral development.

We had hypothesized that the introduction of genes would contribute to increase the inter-plant variability of some traits, especially under crowding stress. Despite the inter-plant variability of traits linked to grain yield (i.e. PGR_{CP} , EGR_{CP} , KNP) increased in response to the most stressful conditions, differences between versions of a hybrid did not show a consistent pattern. Only the Bt-RR version of DK747 group showed higher CVs of EGR_{CP} than the other versions at mean EGR_{CP} values $< 2 \text{ g d}^{-1}$ (Fig. 2a), a documented symptom of the intolerance of a genotype to crowding stress (Tollenaar and Wu, 1999).

5. Conclusions

The introduction of transgenes altered in some way, the development and growth of maize plants in different eco-physiological aspects in the absence of pests, weeds and N deficiency. For example, the DK747RR showed consistently a shorter time to silking than DK747 and the DK747MG, but both DK190RR and DK190MG exhibited a similar duration of this period. Thus, not only the inserted transgene, but also the insertion site and the genetic background could have a role in the expression of observed phenotypic differences. Among growth variables, the stacked transgenic hybrids (DK747MGRR and DK190MGRR) presented the highest ear growth rate around silking, but the lowest reproductive efficiency. To identify the genetic and physiological basis of both traits would be of interest to increase the reproductive success of maize crops.

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