Field Crops Research 191 (2016) 101–110

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Kernel setting at the apical and sub-apical ear of older and newer Argentinean maize hybrids

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ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form 22 February 2016
Accepted 24 February 2016
Available online 12 March 2016

Keywords:
Maize
Kernel number
Apical ear
Sub-apical ear
Biomass partitioning

ABSTRACT

Rainfed maize (*Zea mays*, L.) crops in temperate semi-arid regions with high inter-annual variation of summer precipitation, are commonly cultivated at low population densities. During seasons with favorable conditions for plant growth (e.g., summer rainfalls above normal records), the number of kernels of sub-apical ear can contribute significantly to total kernel number per plant (TKN). However, there is no information of the determinant traits of kernel setting at sub-apical ear, or the effects of breeding on these traits. We used a crop physiology model with an individual plant approach in attempt to describe genotypic differences in those traits related to kernel setting at the apical (KNE_1) and the sub-apical ear (KNE_2) of older and newer Argentinean maize hybrids. Four representative hybrids of the decades of 80′, 90′, 00′ and 10′ were cultivated during two growing seasons at three densities (4, 8 and 12 pl m^{-2}). Non-destructive techniques were used to estimate the growth rate of individual plant (PGR_{PC}), apical (E_1GR_{CP}) and sub-apical (E_2GR_{CP}) ear during the period bracketing silking (critical period), biomass partitioning to reproductive sinks ($E_1GR_{CP}\ PGR_{CP}{}^{-1}$ and $E_2GR_{CP}\ PGR_{CP}{}^{-1}$) and the efficiency to set kernels of the plant (TKN $PGR_{CP}{}^{-1}$) and the ears ($KNE_1\ E_1GR_{CP}{}^{-1}$ and $KNE_2\ E_2GR_{CP}{}^{-1}$). Differences among tested hybrids in KNE_1 (ca. 400–600 k ear^{-1}) were mainly determined by $KNE_1\ E_1GR_{CP}{}^{-1}$ (ca. 230–280 k d^{-1} g^{-1}) while newer hybrids had the highest $E_1GR_{CP}\ PGR_{CP}{}^{-1}$ (>0.50). By contrast, hybrids had similar KNE_2 (ca. 124 k ear^{-1}) due to their similar $E_2GR_{CP}\ PGR_{CP}{}^{-1}$ (ca. 0.08) and $KNE_2\ E_2GR_{CP}{}^{-1}$ (ca. 93 k d^{-1} g^{-1}). These results suggest that breeding effect on TKN was mainly determined by $KNE_1\ E_1GR_{CP}{}^{-1}$ and for the newest hybrid this trait did not interact with plant density. Hence, the newest hybrid exhibited a positive linear response of TKN to PGR_{PC} ($r^2 = 0.82$) reaching the highest TKN among hybrids at low, mid and high density. A better performance of maize crops at low densities could be obtained by breeding for $E_2GR_{CP}\ PGR_{CP}{}^{-1}$.

1. Introduction

Rainfed maize (*Zea mays*, L.) crops in temperate semi-arid regions with high inter-annual variation of summer precipitation (Penalba and Vargas, 2004), are commonly cultivated at low population densities (Berzsenyi and Tokatlidis, 2012). At these densities and during seasons with summer rainfalls above normal records, the number of kernels of sub-apical ear (KNE_2) can contribute significantly to total kernel number per plant (TKN) (Otegui, 1995; Brathwaite and Brathwaite, 2002; Maddonni and Martínez-Bercovich, 2014). However, most hybrids cultivated at these regions, have been the product of a strong selection pressure for high yield in potential environments, where maximum yields are achieved at high plant densities (Tollenaar, 1989; Echarte

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Abbreviations: E_1GR_{CP}, growth rate of apical ear during the critical period; $E_1GR_{CP}\ PGR_{CP}{}^{-1}$, biomass partitioning to apical ear during the critical period; E_2GR_{CP}, growth rate of sub-apical ear during the critical period; $E_2GR_{CP}\ PGR_{CP}{}^{-1}$, biomass partitioning to sub-apical ear during the critical period; Exp., experiment; KNE_1, kernel number of the apical ear; $KNE_1\ E_1GR_{CP}{}^{-1}$, the efficiency of the apical ear to set kernels; KNE_2, kernel number of the sub-apical ear; $KNE_2\ E_2GR_{CP}{}^{-1}$, the efficiency of the sub-apical ear to set kernels; PGR_{CP}, plant growth rate during the critical period; PGR_{CPT}, plant growth rate during the critical period below which $KNE_1 = 0$; TKN, total kernel number per plant; TKN $PGR_{CP}{}^{-1}$, the efficiency of the plant to set kernels.

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http://dx.doi.org/10.1016/j.fcr.2016.02.021
0378-4290/© 2016 Published by Elsevier B.V.

et al., 2000; Sangoi et al., 2002; Duvick, 2005). At these densities, the number of kernels of the apical ear (KNE_1) mainly determines grain yield per unit land area because of the low growth rate of plants during the critical period (PGR_{CP} <3 g d^{-1}) for kernel setting (Andrade et al., 1999; Vega et al., 2001a; Echarte et al., 2004), which involves 30 days bracketing silking. By contrast, maize plants growing at low densities with high soil water contents, attain high PGR_{CP} that enhances the number of fertile ears per plant (i.e., prolificacy > 1), and the contribution of kernels of sub-apical ear to TKN and consequently to crop grain yield (Otegui, 1995; Maddonni and Martínez-Bercovich, 2014).

Several studies have quantified KNE_1 and KNE_2 of older and newer maize hybrids cultivated at contrasting plant densities (Tollenaar et al., 1992; Echarte et al., 2000, 2004; Sangoi et al., 2002). However, only the determinant traits of KNE_1 were studied on a per plant basis using non-destructive techniques (Echarte et al., 2004). Thus, newer hybrids set more KNE_1 than the older ones at high densities (i.e., plants with low PGR_{CP}) due to greater biomass partitioning to the apical ear (ear growth rate per unit of PGR_{CP}; E_1GR_{CP} $PGR_{CP}{}^{-1}$) during the critical period. At low densities (i.e., plants with high PGR_{CP}), the greater KNE_1 of newer hybrids was determined by the greater efficiency of apical ear to set kernels (i.e., high KNE_1 $E_1GR_{CP}{}^{-1}$). Additionally, hyperbolic relationships were used for several authors to compare the relationships KNE_1 vs PGR_{CP} and TKN vs PGR_{CP} of several hybrids. Only threshold values of PGR_{CP} (>4–6 g d^{-1}) for kernel setting at the sub-apical ear were documented, which varied with the potential prolificacy of genotypes (Tollenaar et al., 1992; Tollenaar and Wu, 1999; Andrade et al., 1999; Vega et al., 2001a,b). However, differences in determinant traits of KNE_2 (i.e., E_2GR_{CP} $PGR_{CP}{}^{-1}$ and KNE_2 $E_2GR_{CP}{}^{-1}$) among current maize hybrids and between older and newer hybrids were never reported. The study of those traits related to KNE_2 could improve our knowledge of maize performance under "defensive management strategies" (e.g. cultivation at low plant densities) commonly used in areas with erratic rainfall distribution, that aim stabilize harvested yields at the expense of potential yields (Popp et al., 2006; Birch et al., 2008; Tokatlidis et al., 2011; Berzsenyi and Tokatlidis, 2012).

In this work four commercial maize hybrids released during the last decades in Argentina were cultivated in two field experiments at three plant densities (low, mid and high density). We used a crop physiology model with an individual plant approach in attempt to describe genotypic differences in those traits related to KNE_1 and KNE_2 of older and newer maize hybrids.

2. Materials and methods

2.1. *Crop management and experimental design*

The experiments were conducted at the experimental field of the Department of Plant Production, University of Buenos Aires, Argentina (35′S 34th, 58th 29′W) on a deep silty clay loam soil (Vertic Argiudoll; Soil Survey Staff, 2010) during 2012–2013 and 2013–2014. Four Argentinean maize hybrids representative of the 80′, 90′, 00′ and 10′ decades (1983: DK-3F22, 1993: DK-752, 2004: DK-747 and 2012: DK-7210) were cultivated at three plant densities (4, 8 and 12 plants m^{-2}) in a split plot design with three replicates. These hybrids were selected because of their commercial importance in the area under study. After their release, all selected genotypes were among the topmost cropped hybrids for at least 5 years. Plant densities were assigned to the main plots and hybrids to the subplots. Each subplot involved three rows, 4 m long, spaced at 0.70 m.

The experiments were manually sown on November 29, 2012 (Exp_1) and October 22, 2013 (Exp_2) to explore a wide range of climatic conditions around silking (Fig. 1). To ensure the stand of plants, three seeds per hill were sown and later thinned to one plant per site at the three-ligulated-leaf stage (V_3; Ritchie et al., 1993). The experiments were fertilized with di-ammonium phosphate at sowing and with urea at V_{4-6} totaling a dose of 150 kg N ha^{-1}. Weeds, pests and diseases were chemically and mechanically controlled. Soil moisture remained close to field capacity throughout the crop cycle by using drip irrigation. Although the variability of water availability is characteristic of temperate semi-arid regions, we used irrigation because our main objective was to determine kernel setting at both ears for plants with different PGR_{CP} (promoted by combinations of plant densities and weather conditions). Moreover, PGR_{CP} taken as an indicator of the amount of carbon available to the plants, is a good predictor of the capacity of maize plants to set kernels under a wide range of environmental conditions (water deficiencies, N deficiencies, shading) and management practices (Andrade et al., 2002).

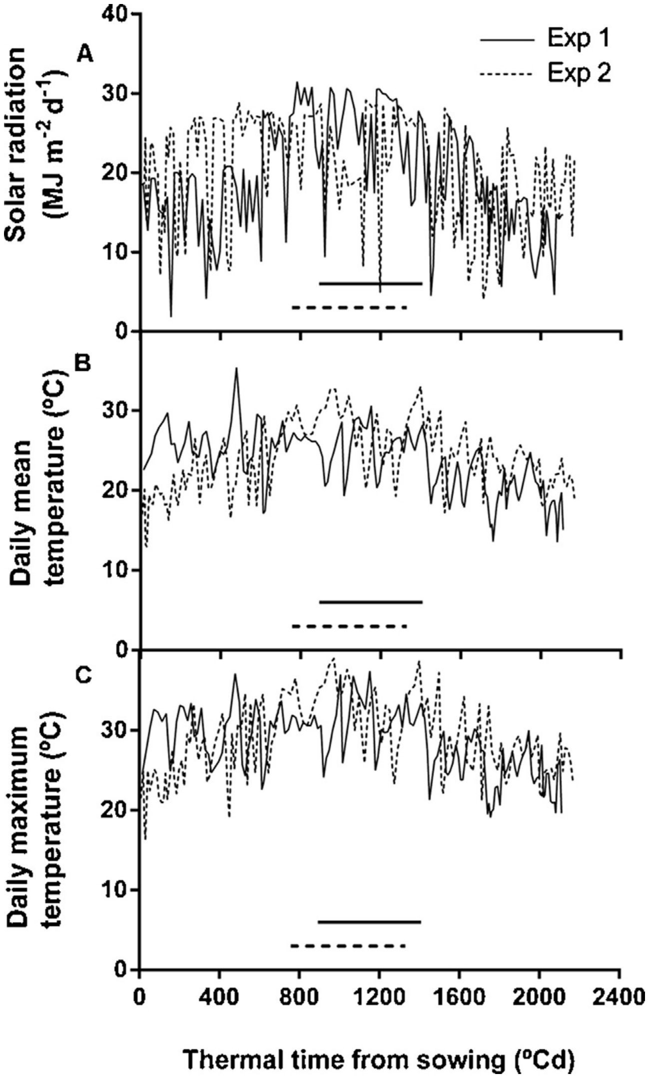

Fig. 1. Solar radiation (A), mean air temperature (B) and maximum temperature (C) during two growing seasons (solid lines: Exp_1, dotted lines: Exp_2). Time from sowing is expressed in thermal time units (°C d) with a base temperature of 8 °C. The horizontal lines represent the extension of the critical period of tested hybrids in each experiment.

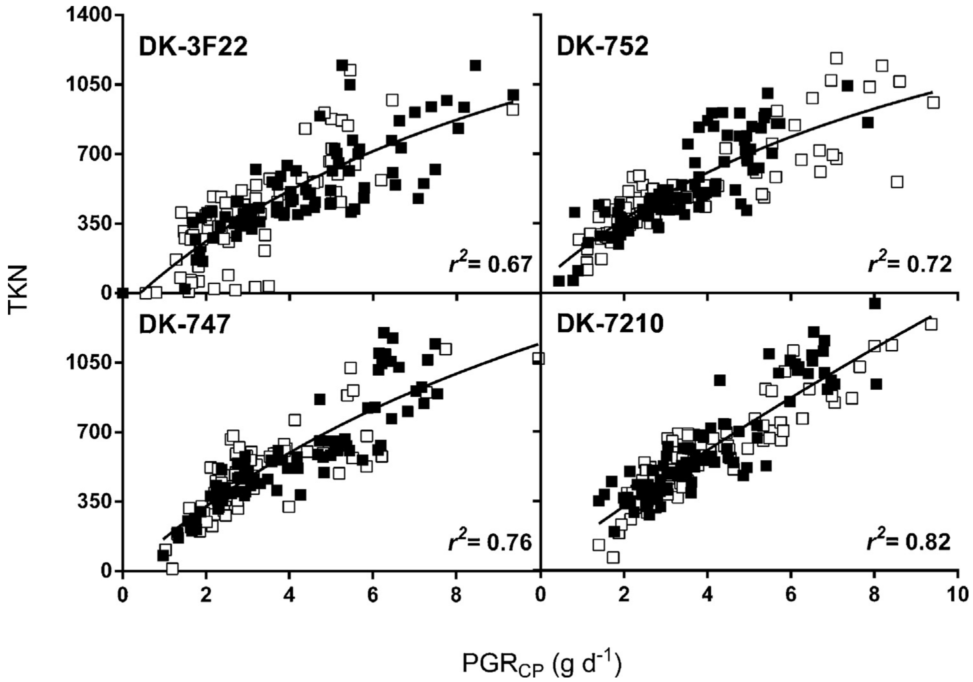

Fig. 2. Relationship between kernel number per plant (TKN) and plant growth rate during the critical period (PGR_{CP}) of four maize hybrids cultivated at three plant densities in two experiments. Each symbol identifies a plant (black symbols: data of Exp_1, white symbols: data of Exp_2). The lines represent the curvilinear function fitted to data set of each hybrid. The coefficients of determination are detailed.

2.2. Measurements

Ten consecutive plants of equal size and similar ontogenic stage were tagged at V_3 in the middle row of each subplot. For each plant, the number of ligulated leaves (V_n stages) was recorded weekly. Daily observations of silk exsertion from the apical and sub-apical ear of each tagged plant were performed and the silking dates of these ears were recorded when at least one silk per ear was visible. Non-destructive allometric models were used to estimate vegetative biomass of each tagged plant at (i) ca. 15 days before silking date of the apical ear, (ii) silking of the apical ear and (iii) 15 days after silking date of the apical ear. Measurements involved maximum and minimum stem base diameter and plant height from the ground level to the insertion of the last expanded leaf. Allometric models were also used to estimate biomass of the apical and sub-apical ear of each tagged plant at the silking date of each ear and 15 days after that date using measurements of the maximum diameter of the ears. Total plant biomass at silking date of the apical ear and 15 days after that date was computed as the sum of estimated vegetative biomass and those of the apical and the sub-apical ear. This non-destructive technique has been widely used in maize to estimate PGR_{CP} and E_1GR_{CP} of tagged plants remaining in the field until harvest (Vega et al., 2001b; Echarte et al., 2004; Pagano and Maddonni, 2007; Rossini et al., 2011; Laserna et al., 2012). This methodology was never used before to estimate E_2GR_{CP}.

During Exp_1 and Exp_2, allometric models were built with plants of each hybrid (n > 20 plants per hybrid × density × Exp) sampled along the pre- and post-silking period. Measurements described above were performed on these sampled plants. Vegetative biomass (stalk + leaves + tassel) was estimated based on linear or bi-linear functions fitted to total vegetative dry weight and stem volume (Table 1) considering theequation of cylinder volume (Eq. (1)).

$$V = \pi \times \left(\frac{d}{2}\right)^2 \times h \tag{1}$$

where d is the average diameter of the base of the stem (cm) and h is the stem height (cm).

Biomass of the apical and sub-apical ear (husks + cob + florets) at their silking dates and 15 days after those dates was estimated with an exponential function fitted to ear dry weight and maximum ear diameter (mm) of sampled ears of each genotype (Table 1).

At physiological maturity tagged plants were individually harvested and prolificacy (ears per plant with more than ten kernels; Tollenaar et al., 1992), KNE_1 and KNE_2 were counted.

Table 1
Parameters of allometric models (detailed at the bottom of the table) used for estimating vegetative biomass (stems + leaves + tassels) and biomass of apical and sub-apical ears. For each model the coefficient of determination (r^2) and the number (n) of sampled plants are detailed.

	Vegetative biomass						Apical/sub-apical ear biomass			
Hybrid	*a*	*b*	*c*	*d*	r^2	*n*	*e*	*f*	r^2	*n*
DK-3F22	13.5	0.15			0.92	141	1.43	0.07	0.92	132
DK-752	20.6	0.13			0.89	142	0.76	0.09	0.92	156
DK-747	9.8	0.14	886.6	0.06	0.86	146	1.29	0.08	0.93	128
DK-7210	12.8	0.13	1017.0	0.04	0.85	141	1.72	0.07	0.90	139

For vegetative biomass = $a + b$ Vol (cm^3), or $a + b$ Vol if Vol < = c and $a + b\,c + d$ (Vol-c) if Vol > c where Vol = is the stem volume estimated with Eq. (1).
For ear biomass = $e^{f\ \text{ear diameter(mm)}}$.

Table 2
Growth rate of plant (PGR_{CP}), apical ear (E_1GR_{CP}) and sub-apical ear (E_2GR_{CP}) during the critical period, the number of kernels per plant (TKN), per apical ear (KNE_1) and per sub-apical ear (KNE_2) and prolificacy of four maize hybrids (H) grown at three plant densities (D) [4 (D_4), 8 (D_8) and 12 (D_{12}) plants m^{-2}] in two field experiments (Exp).

		PGR_{CP}	E_1GR_{CP} ($g\,d^{-1}$)	E_2GR_{CP}[a]	TKN	KNE_1	KNE_2[b]	Prolificacy ears pl^{-1}
Experiment	1	4.01 b	1.96 b	0.49 b	565.8 b	476.8 b	135.1	1.26
	2	3.70 a	1.69 a	0.38 a	522.5 a	455.9 a	113.5	1.21
	p	*	***	*	*	*	ns	ns
Density	D_4	5.82 c	2.42 c	1.11 c	807.8 c	577.4 c	327.2 a	1.70 b
	D_8	3.51 b	1.83 b	0.16 b	487.3 b	482.3 b	43.0 b	1.04 a
	D_{12}	2.24 a	1.24 a	0.04 a	337.3 a	339.4 a	2.8 c	0.98 a
	p	***	***	***	***	***	***	***
Hybrid	DK-3F22	3.80 ab	1.64 a	0.43	466.5 a	399.6 a	158.7 b	1.17 a
	DK-752	3.64 a	1.84 b	0.46	536.0 b	447.4 b	114.1 a	1.31 b
	DK-747	3.88 ab	1.91 b	0.38	559.8 b	492.8 c	109.0 a	1.20 a
	DK-7210	4.12 b	1.92 b	0.48	614.2 c	525.6 d	115.4 ab	1.26 ab
	p	†	***	ns	***	***	†	*
Exp × D	p	ns	*	*	ns	ns	ns	†
Exp × H	p	**	ns	ns	ns	**	ns	ns
H × D	p	ns	ns	ns	ns	ns	ns	*
Exp × H × D	p	†	ns	ns	ns	ns	ns	ns

Within each column, different letters indicate significant differences at $p < 0.05$. The significance level of treatments and interactions on each trait are indicated by symbols: † $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns: no significant difference.

[a] Mean values of E_2GR_{CP}, involved plants with $E_2GR_{CP} \geq 0$.

[b] Mean values of KNE_2, involved plants with $KNE_2 > 10$.

2.3. Functional relationships and data analysis

The different strategies for kernel setting of tested hybrids were explored based on their determinant traits. At the individual plant level, TKN is the sum of KNE_1 and KNE_2. Both KNE_1 and KNE_2 depend on the growth of apical and sub-apical ear respectively, around their silking (i.e., E_1GR_{CP} and E_2GR_{CP}) and in the efficiency of this growth to set kernels (i.e., $KNE_1\ E_1GR_{CP}^{-1}$ and $KNE_2\ E_2GR_{CP}^{-1}$). The growth of the ears depends on PGR_{CP} and in the biomass partitioning to these organs (i.e., $E_1GR_{CP}\ PGR_{CP}^{-1}$ and $E_2GR_{CP}\ PGR_{CP}^{-1}$). The PGR_{CP} of each tagged plant was estimated from the slope of the linear regression fitted to estimated plant biomass at three stages (ca. 15 days before silking date of the apical ear, silking date of the apical ear and 15 days after the silking date of the apical ear) and time in days from sowing. Similarly, E_1GR_{CP} and E_2GR_{CP} were estimated from the slopes of the linear regressions fitted to estimated ear biomass at (i) ca. 15 days before the silking date of the apical ear (i.e., ear biomass close to zero; Otegui and Bonhomme, 1998), (ii) the silking date of each ear and (iii) 15 days after these dates and time after sowing. For plants with no silk exsertion at the sub-apical ear, E_2GR_{CP} was computed as zero. Biomass partitioning to ears was estimated through the ratio between E_1GR_{CP} or E_2GR_{CP} and PGR_{CP} (Vega et al., 2001b; Pagano and Maddonni, 2007; Laserna et al., 2012). Finally, the ratio between TKN and PGR_{CP} (TKN PGR_{CP}^{-1}) was used to analyze the efficiency in kernel setting at the plant level. Similarly $KNE_1\ E_1GR_{CP}^{-1}$ and $KNE_2\ E_2GR_{CP}^{-1}$ were calculated to estimate the efficiency in kernel setting of each ear (Vega et al., 2001b; Laserna et al., 2012). For $KNE_2\ E_2GR_{CP}^{-1}$, only plants with $KNE_2 > 10$ were computed. For each genotype a hyperbolic function was fitted to TKN vs PGR_{CP}, KNE_1 vs PGR_{CP}, KNE_1

Table 3
Parameters (values and confidence limit at $p < 0.05$) of the curvilinear function fitted to the relationships between: i) the total kernel number per plant (TKN) and plant growth rate during the critical period (PGR_{CP}), ii) kernel number per apical ear (KNE_1) and PGR_{CP}, iii) KNE_1 and growth rate of apical during the critical period (E_1GR_{CP}) and iv) kernel number per sub-apical ear (KNE_2) and the growth rate of the sub-apical ear during the critical period (E_2GR_{CP}) of four maize hybrids grown at three densities in two field experiments. The curvilinear function is detailed at the bottom of the table. For each function the coefficient of determination and the number (n) of plants are detailed. Below each relationship the p value for the rejection on null hypothesis (one curve for all hybrids) is detailed.

		Parameters			
	Hybrids	a	b	Threshold	r^2; n
TKN vs PGR_{CP}	DK-3F22	186.5 ± 78.1	0.08 ± 0.09	0.42 ± 0.65	0.67; 160
($p < 0.001$)	DK-752	198.4 ± 76.9	0.09 ± 0.08	−0.26 ± 0.68	0.72;164
	DK-747	192.8 ± 76.6	0.07 ± 0.06	0.09 ± 0.70	0.76; 161
	DK-7210	156.4 ± 59.2	0.02 ± 0.05	−0.15 ± 1.11	0.82; 168
KNE_1 vs PGR_{CP}	DK-3F22	434.2 ± 211.4	0.60 ± 0.40	0.96 ± 0.39	0.57; 153
($p < 0.001$)	DK-752	627.5 ± 216.8	0.97 ± 0.42	0.47 ± 0.25	0.66; 157
	DK-747	710.5 ± 161.6	0.93 ± 0.27	1.11 ± 0.15	0.75; 160
	DK-7210	558.5 ± 183.6	0.66 ± 0.28	1.01 ± 0.31	0.67; 168
KNE_1 vs E_1GR_{CP}	DK-3F22	1014 ± 374.3	1.34 ± 0.70	0.55 ± 0.11	0.66; 157
($p < 0.001$)	DK-752	1087 ± 310.3	1.54 ± 0.58	0.45 ± 0.10	0.71; 165
	DK-747	1003 ± 235	1.18 ± 0.39	0.52 ± 0.09	0.74; 161
	DK-7210	1802 ± 656	2.32 ± 1.08	0.80 ± 0.10	0.59; 168
KNE_2 vs E_2GR_{CP}	DK-3F22	1122.0 ± 867	2.05 ± 2.39	0.77 ± 0.12	0.77; 80
($p < 0.001$)	DK-752	365.0 ± 131	0.13 ± 0.29	0.39 ± 0.11	0.90; 93
	DK-747	699.0 ± 390	0.79 ± 0.88	0.68 ± 0.11	0.88; 60
	DK-7210	318.9 ± 107	−0.05 ± 0.19	0.48 ± 0.13	0.92; 82

$y = a(x - \text{threshold})/1 + b(x - \text{threshold})$ for $x >$ threshold.

Table 4
Biomass partitioning to the apical ear (E_1GR_{CP} PGR_{CP}^{-1}) and to sub-apical ear (E_2GR_{CP} PGR_{CP}^{-1}) during the critical period and the efficiency to set kernels at the plant level (TKN PGR_{CP}^{-1}), apical ear level (KNE_1 $E_1GR_{CP}^{-1}$) and sub-apical ear level (KNE_2 $E_2GR_{CP}^{-1}$) of four maize hybrids (H) grown at three plant densities (D) [4 (D_4), 8 (D_8) and 12 (D_{12}) plants m^{-2}] in two field experiments (Exp).

		E_1GR_{CP} PGR_{CP}^{-1}	E_2GR_{CP} PGR_{CP}^{-1}	TKN PGR_{CP}^{-1}	KNE_1 $E_1GR_{CP}^{-1}$ (kernels g^{-1} d^{-1})	KNE_2 $E_2GR_{CP}^{-1}$
Experiment	1	0.51	0.09 b	143.0	244.6 a	98.7
	2	0.49	0.07 a	146.8	272.4 b	87.3
	p	ns	**	ns	***	ns
Density	D_4	0.43 a	0.19 c	142.4	243.9 a	229.3 c
	D_8	0.53 b	0.04 b	144.4	268.3 b	46.1 b
	D_{12}	0.55 b	0.02 a	147.9	263.3 b	3.8 a
	p	***	***	ns	**	***
Hybrid	DK-3F22	0.44 a	0.08 ab	118.8 a	233.0 a	116.5
	DK-752	0.56 c	0.09 b	160.8 c	257.2 b	92.0
	DK-747	0.51 b	0.07 a	147.8 b	262.0 b	76.4
	DK-7210	0.50 b	0.08 ab	152.1 bc	281.9 c	87.3
	p	***	†	***	***	ns
Exp × D	*p*	ns	*	**	†	ns
Exp × H	*p*	***	*	ns	*	ns
H × D	*p*	**	ns	*	**	ns
Exp × H × D	*p*	ns	ns	ns	ns	ns

Within each column, different letters indicate significant differences at $p < 0.05$. The significance level of treatments and interactions on each trait are indicated as: † $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns: no significant difference.

vs E_1GR_{CP} and KNE_2 vs E_2GR_{CP} based on the curvilinear function proposed by Vega et al. (2001a) for KNE_1 vs PGR_{CP} (Eq. (2)).

$$KNE_1 = \frac{a(PGR_{CP} - PGR_{CPT})}{1 + b(PGR_{CP} - PGR_{CPT})}, \quad \text{for } PGR_{CP} > PGR_{CPT} \qquad (2)$$

where *a* is the initial slope (indicative of the efficiency in kernel setting at low PGR_{CP}), PGR_{CPT} is the threshold value of PGR_{CP} below which $KNE_1 = 0$ (i.e., a threshold for plant sterility) and *b* is the coefficient of the curvi-linearity of the hyperbolic function at high PGR_{CP} (i.e., the inverse of the efficiency in kernel setting at high PGR_{CP}).

Particularly for KNE_2 vs E_2GR_{CP}, plants with positive values of E_2GR_{CP} were used to fit the curvilinear model (Eq. (2)). Thus, plants with KNE_2 equal to 0 but with $E_2GR_{CP} > 0$ were useful to estimate E_2GR_{CPT}.

The effects of the experiment, plant density, hybrid and their interactions on measured traits were evaluated by analysis of variance (ANOVA), based on the corresponding error sources of the subplot design. The experiment was included as a class variable. An ANOVA was performed for each experiment to explore density × hybrid interaction for all traits within each experiment. Fischer LSD test was used to establish the existence of significant differences among mean values. Functions fitted to the relationships between traits were performed with GradPadPrism (GraphPadPrism 6.00 for Windows), and the null hypothesis of one curve for all hybrids was tested with an alpha = 0.05. A confidence interval of 95% was used for estimating significant differences among hybrids in the parameters of fitted functions. A correlation matrix was used to explore linear correlations among traits.

3. Results

3.1. Description of the weather conditions during the experiments

Total solar radiation during Exp_1 was 6.6% lower than during Exp_2 (Fig. 1A). Similarly, mean air temperature was slightly lower in Exp_1 (23.2 °C) than in Exp_2 (23.5 °C) (Fig. 1B). The critical period of hybrids in Exp_1 lasted from mid-January to mid-February, while the same period in Exp_2 lasted from mid-December to mid-January. Thus, during this period, crop in Exp_1 was exposed to lower mean air temperatures (ca. 25.2 °C vs 27.1 °C for Exp_1 and Exp_2; respectively), higher (8.4%) incident solar radiation values and less

Table 5
Parameters (values and confidence limit at $p < 0.05$) of the functions fitted to the relationship between the growth rate of apical (E_1GR_{CP}) and sub-apical ears (E_2GR_{CP}) and plant growth rate (PGR_{CP}) during the critical period of four maize hybrids grown at three population densities. The functions are detailed at the bottom of the table. For each function the coefficient of determination and the number (n) of plants are detailed. Below each relationship the *p* value for the rejection on null hypothesis (one model for all hybrids) is detailed.

	Hybrid	Parameters *a*	*b*	r^2; *n*
E_1GR_{CP} vs PGR_{CP} ($p < 0.001$)	DK-3F22	0.51 ± 0.16	0.31 ± 0.04	0.62; 158
	DK-752	0.47 ± 0.13	0.40 ± 0.04	0.76; 152
	DK-747	0.33 ± 0.11	0.42 ± 0.03	0.85; 160
	DK-7210	0.68 ± 0.17	0.31 ± 0.03	0.66; 157

	Hybrid	Parameters *a*	*b*	*c*	*d*	r^2. *n*
E_2GR_{CP} vs PGR_{CP} ($p < 0.001$)	DK-3F22	−0.04 ± 0.16	0.06 ± 0.14	3.2 ± 0.81	0.31 ± 0.05	0.62; 158
	DK-752	−0.01 ± 0.13	0.02 ± 0.13	2.7 ± 0.43	0.41 ± 0.06	0.70; 152
	DK-747	−0.03 ± 0.11	0.05 ± 0.07	4.6 ± 0.39	0.60 ± 0.10	0.72; 160
	DK-7210	−0.07 ± 0.13	0.08 ± 0.09	4.0 ± 0.38	0.54 ± 0.07	0.80; 157

For E_1GR_{CP} vs PGR_{CP}, $E_1GR_C = a + b\ PGR_{CP.}$
For E_2GR_{CP} vs PGR_{CP}, $E_2GR_{CP} = a + b\ PGR_{CP}$ if $PGR_{CP} < c$ and $E_2GR_{CP} = a + b\ c + d\ PGR_{CP}$ if $PGR_{CP} > c$.

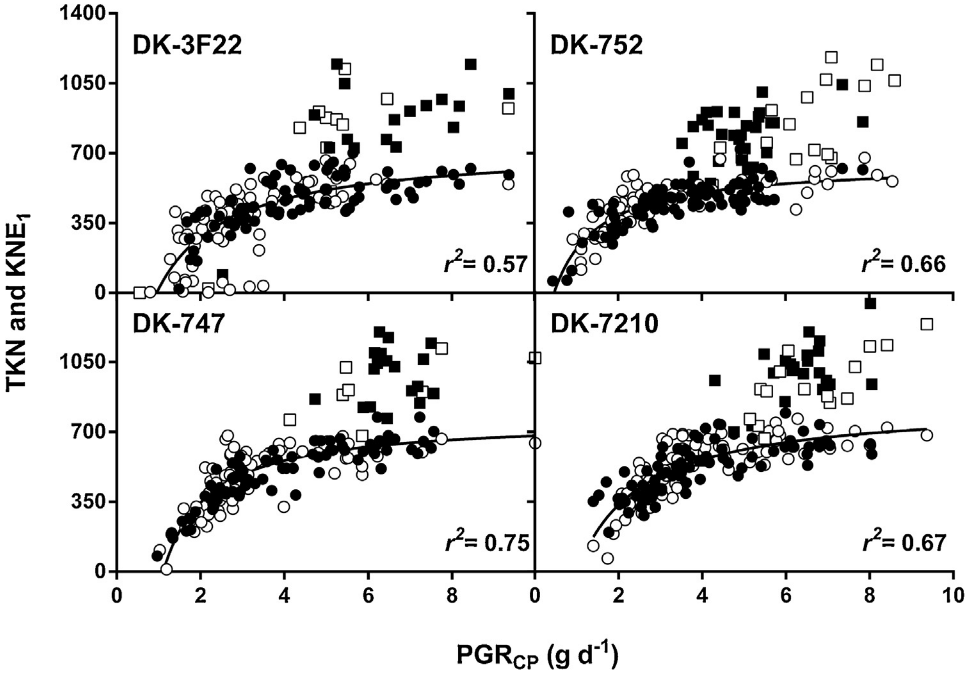

Fig. 3. Relationship between total kernel number per plant (TKN) and kernel number of apical ear (KNE_1) with plant growth rate during the critical period (PGR_{CP}) of four maize hybrids cultivated at three plant densities in two experiments (full symbols: Exp_1, empty symbols: Exp_2). Each symbol identifies TKN (squares) and KNE_1 (circles) of a plant. The lines represent the curvilinear function fitted to KNE_1 vs PGR_{CP} of each genotype. The coefficients of determination are detailed.

days with maximum temperature above 35 °C (i.e., less heat stress events) (Fig. 1C) than in Exp_2.

3.2. Plant and ear growth rate and kernel setting

The different environmental conditions of the experiments were reflected in several plant traits. Thus, PGR_{CP} in Exp_1 was higher ($p < 0.05$) than in Exp_2 and this trait decreased ($p < 0.001$) in response to the increased number of plants per unit area (Table 2). Hybrids only differed in PGR_{CP} at the lowest density with a different ranking order between experiments ($p < 0.10$ for experiment × hybrid × density interaction). At this density, DK-752 in Exp_1 and DK-747 in Exp_2 exhibited the lowest PGR_{CP} (Supplemental Table 2). No significant experiment × density or hybrid × density interaction was detected for PGR_{CP} (Table 2).

Significant ($0.10 < p < 0.05$) experiment x density interactions were detected for E_1GR_{CP}, E_2GR_{CP} and prolificacy (Table 2). At the lowest density, E_1GR_{CP} did not differ between experiments, but at mid and high density E_1GR_{CP} was higher in Exp_1 than in Exp_2 (Supplemental Table 2). By contrast, E_2GR_{CP} (ca. 1.24 and 0.98 g d^{-1} in Exp_1 and Exp_2; respectively) and prolificacy (ca. 1.78 and 1.62 ears pl^{-1} in Exp_1 and Exp_2; respectively) only differed between experiments at the lowest density (Supplemental Table 2). In both experiments and at all densities, the oldest hybrid (DK-3F22) had the lowest ($p < 0.001$) E_1GR_{CP} while E_2GR_{CP} was similar among genotypes (Table 2). Differences among hybrids in prolificacy were only recorded at the lowest density ($p < 0.05$ for hybrid × density interaction, Supplemental Table 2). At this density, DK-7210 (ca. 1.77 ears pl^{-1}) and DK-752 (ca. 1.88 ears pl^{-1}) exhibited greater prolificacy than DK-747 (ca. 1.59 ears pl^{-1}) and DK-3F22 (ca. 1.55 ears pl^{-1}).

A significant experiment × hybrid interaction ($p < 0.01$) was detected for KNE_1 (Table 2). In Exp_1, DK-747 and DK-7210 attained higher KNE_1 than DK-3F22 and DK-752 (Supplemental Table 2). By contrast, in Exp_2 KNE_1 differed among all hybrids and this trait increased with the year of release. Plant density affected KNE_2 ($p < 0.001$) and DK-3F22 had a higher KNE_2 ($p < 0.10$) than DK-752 and DK-747 (Table 2).

In both Exps, TKN decreased ($p < 0.001$) as plant density was increased and at all densities DK-3F22 exhibited the lowest ($p < 0.001$) TKN, DK-752 and DK-747 intermediate TKN, and DK-7210 the highest TKN (Table 2 and Supplemental Table 2).

Total kernel number per plant positively responded to changes in PGR_{CP} caused by treatments, and the curvilinear function explained more than 67% of TKN variability of each hybrid (Table 3 and Fig. 2). Differences among hybrids ($p < 0.001$) were detected in the fitted curves (Table 3). The oldest hybrid was the only genotype that produced sterile plants when PGR_{CP} was lower than 4 g d^{-1}. For the newest hybrid, a linear function ($TKN = 133.5\ PGR_{PC} + 65.29$) described TKN response to PGR_{CP} with a coefficient of determination ($r^2 = 0.82$) similar to that of the curvilinear function (Fig. 2).

For all hybrids, the best fit between KNE_1 and PGR_{CP} was obtained with the curvilinear function that explained more than 57% of KNE_1 variability (Table 3 and Fig. 3). Fitted curves differed ($p < 0.001$) among hybrids. Kernel setting at the sub-apical ear of all hybrids occurred when $PGR_{CP} > 4$ g d^{-1} but most plants of DK-7210 exhibited fertile sub-apical ears when $PGR_{CP} > 6$ g d^{-1} (Fig. 3).

3.3. Biomass partitioning to reproductive organs and kernel set efficiency

The $E_1GR_{CP}\ PGR_{CP}^{-1}$ did not differ between experiments but $E_2GR_{CP}\ PGR_{CP}^{-1}$ in Exp_1 was higher ($p < 0.01$) than in Exp_2 (Table 4). The oldest hybrid (DK-3F22) had the lowest $E_1GR_{CP}\ PGR_{CP}^{-1}$ in Exp_1, but similar to that of DK-7210 in Exp_2 ($p < 0.001$ for Exp × hybrid interaction, Table 4 and Supplemental Table 4). By contrast, the highest $E_2GR_{CP}\ PGR_{CP}^{-1}$ was recorded for DK-752 in Exp_1 at the lowest density ($p < 0.001$ for experiment × density interaction, Table 4 and Supplemental Table 4). The $E_1GR_{CP}\ PGR_{CP}^{-1}$ of DK-752, DK-747 and DK-7210 increased in response to increased plant density while that of DK-3F22 was not affected by crowding ($p < 0.01$ for hybrid × density interaction, Table 4 and Supplemental Table 4). By contrast, $E_2GR_{CP}\ PGR_{CP}^{-1}$ of all hybrids decreased

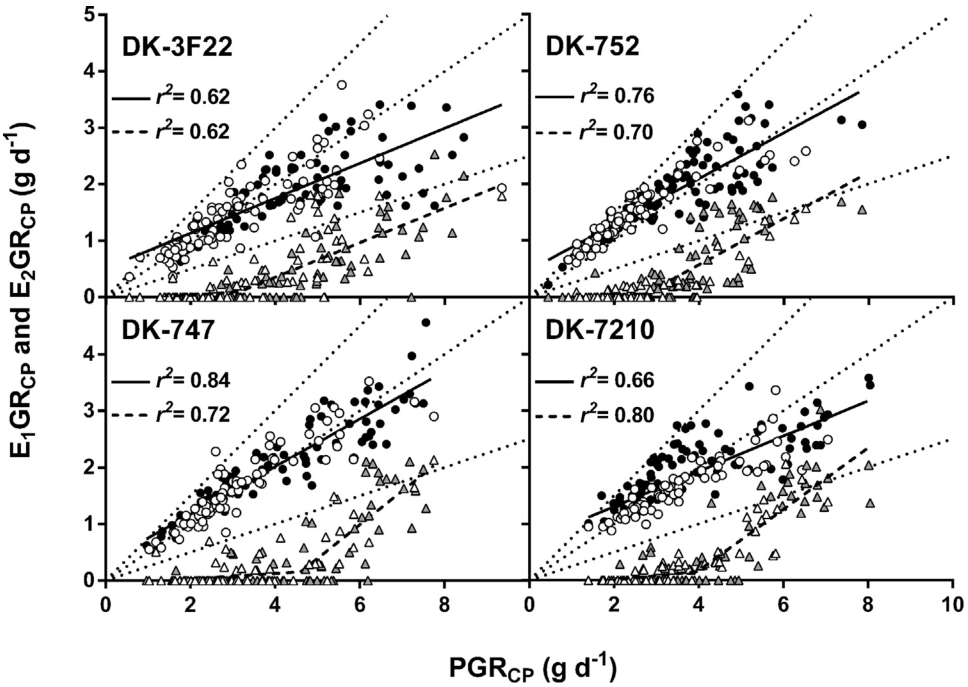

Fig. 4. Relationships between the growth rate of apical (E_1GR_{CP}) and sub-apical ear (E_2GR_{CP}) with plant growth rate during the critical period (PGR_{CP}) of four maize hybrids cultivated at three plant densities in two experiments (full symbols: Exp_1, empty symbols: Exp_2). Each symbol identifies the growth of apical (circles) or sub-apical (triangles) ear of a plant. The lines represent the linear (solid line) or bi-linear (dashed line) functions fitted to data set of apical and sub-apical ear of each hybrid. Dotted lines represent equal quotients between $E_{1\text{-}2}GR_{CP}$ and PGR_{CP} (i.e., biomass partitioning to ears) of 0.25, 0.50 and 0.75.

($p < 0.001$) in response to increasing plant density, but with a high magnitude order in Exp_2 ($p < 0.05$ for experiment x density interaction, Table 4 and Supplemental Table 4) than in Exp_1.

A linear function adequately described the relationships between E_1GR_{CP} and PGR_{CP} for each hybrid ($r^2 > 0.62$) (Fig. 4). The slopes of DK-752 and DK-747 were the highest (i.e., higher E_1GR_{CP} response to PGR_{CP}) ($p < 0.05$) (Table 5) and linear regressions fitted to DK-752 and DK-747 were close to the line of 0.50 biomass partitioning to E_1. By contrast, a bi-linear model suitably described the relationship between E_2GR_{CP} and PGR_{CP} ($r^2 > 0.62$) (Fig. 4 and Table 5). For all hybrids, the ordinate and the first slope of the function did not differ from zero (i.e., biomass partitioning to E_2 was negligible) up to PGR_{CP} greater than ca. 3.1 (DK-3F22 and DK-752) or 4.3 g d^{-1} (DK-747 and DK-7210). Above these PGR_{CPT} values, plants exhibited a steeper E_2GR_{CP} response to PGR_{CP}. This second slope was higher ($p < 0.05$) in the newer hybrids (DK-747 and DK-7210) and some plants exhibited E_2GR_{CP} above the line of 0.25 biomass partitioning to E_2 (Fig. 4). By contrast, almost all plants of the oldest hybrid (DK-3F22) showed E_2GR_{CP} below the 0.25 line of biomass partitioning to E_2.

The highest TKN $PGR_{CP}{}^{-1}$ was recorded at the lowest density in Exp_1 and at mid-density in Exp_2 ($p < 0.01$ for experiment × density interaction, Table 4 and Supplemental Table 4). The TKN $PGR_{CP}{}^{-1}$ response to plant density differed among hybrids ($p < 0.05$ for hybrid × density interaction, Table 4 and Supplemental Table 4). The TKN $PGR_{CP}{}^{-1}$ of the oldest hybrid decreased at mid and high densities. By contrast, TKN $PGR_{CP}{}^{-1}$ of DK-752 exhibited a positive response to the number of plants per unit area. The newer hybrids (DK-747 and DK-7210) exhibited similar TKN $PGR_{CP}{}^{-1}$ at all tested densities.

The KNE_1 $E_1GR_{CP}{}^{-1}$ was higher ($p < 0.001$) in Exp_2 than in Exp_1 (Table 4). In Exp_1, KNE_1 $E_1GR_{CP}{}^{-1}$ was similar among densities and hybrids. By contrast, in Exp_2 the lowest value of this trait was recorded at the lowest density ($p < 0.1$ for experiment x density interaction, Table 4 and Supplemental Table 4) and for the oldest hybrid ($p < 0.05$ for experiment x hybrid interaction, Table 4 and Supplemental Table 4). For DK-3F22 and DK-7210, KNE_1 $E_1GR_{CP}{}^{-1}$ did not change with plant density while for the other hybrids, KNE_1 $E_1GR_{CP}{}^{-1}$ increased in response to higher densities ($p < 0.01$ for hybrid × density interaction, Table 4 and Supplemental Table 4).

The KNE_2 $E_2GR_{CP}{}^{-1}$ did not differ between experiments and hybrids (Table 4). For all hybrids the highest ($p < 0.001$) KNE_2 $E_2GR_{CP}{}^{-1}$ was recorded at the lowest density.

Treatment effects on PGR_{CP}, E_1GR_{CP} $PGR_{CP}{}^{-1}$ and E_2GR_{CP} $PGR_{CP}{}^{-1}$ determined a wide range of E_1GR_{CP} and E_2GR_{CP}. Kernel setting at each ear was related to the growth of these reproductive organs and curvilinear functions were fitted to data set of each ear and hybrid (Fig. 5 and Table 3). At the lowest density, and for similar values of E_1GR_{CP} and E_2GR_{CP}, KNE_1 was slightly higher than KNE_2, showing similar efficiencies for kernel setting of both structures (Supplemental Table 4). Furthermore, hybrids differed ($p < 0.001$) in the KNE_1 vs E_1GR_{CP} relationship, especially in the threshold value of E_1GR_{CP} below which barrenness occurs (Table 3). This threshold was greater than zero for all hybrids and DK-7210 exhibited the highest value. The curvilinear function fitted to KNE_2 vs E_2GR_{CP} also differed among hybrids ($p < 0.001$) showing a positive threshold value of E_2GR_{CP} above which plant set kernels at sub-apical ear (Table 3). Threshold values of DK-3F22 and DK-747 were greater ($p < 0.05$) than those of DK-752 and DK-7210.

4. Discussion

During favorable growing seasons, maize hybrids cultivated at low densities may set kernels at the sub-apical ear increasing grain yield performance (Brathwaite and Brathwaite, 2002; Maddonni and Martínez-Bercovich, 2014). However, most current hybrids, widely cultivated at different locations of maize regions, were selected under plant population densities that suppress the expression of a fertile sub-apical ear (Tollenaar and Lee, 2002). We used a crop physiology model with an individual plant approach in attempt to perform a retrospective analysis of those traits related to KNE_1 and KNE_2 of four maize Argentinean hybrids released in

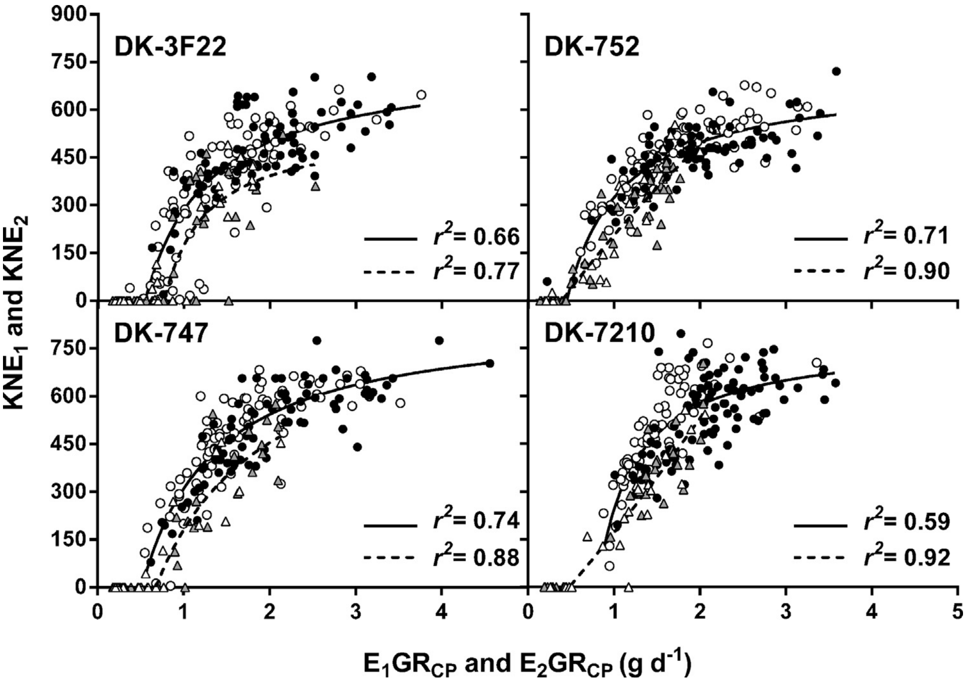

Fig. 5. Relationships between kernel number of apical (KNE$_1$) and sub-apical (KNE$_2$) ear and the growth rate of each ear (E$_1$GR$_{CP}$ and E$_2$GR$_{CP}$) during the critical period of four maize hybrids cultivated at three plant densities in two experiments (full symbols: Exp$_1$, empty symbols: Exp$_2$). Each symbol identifies KNE$_1$ (circles) and KNE$_2$ (triangles) of a plant. The lines represent the curvilinear functions fitted to data set of KNE$_1$ (solid line) and KNE$_2$ (dashed line) of each hybrid.

different decades. The newest hybrid (DK-7210; released in 2012), exhibited a linear response of TKN to PGR$_{CP}$ due to its highest TKN both at low PGR$_{CP}$ (i.e., high density) and high PGR$_{CP}$ (i.e., low density) (Supplemental Table 2). By contrast, the oldest hybrid (DK-3F22; released in 1983), exhibited several sterile plants when PGR$_{CP}$ < 4 g d^{-1} and a curvilinear response of TKN to PGR$_{CP}$. Thus, plant breeding would have improved simultaneously potential yield per plant (i.e., TKN at low density) and the tolerance to crowding stress (i.e., TKN at high density) in contrast to results reported by Echarte et al. (2004) and Tollenaar and Lee (2002). These statements are evidenced not only for the highest TKN of the newest hybrid at contrasting plant densities, but also for its great reproductive plasticity. The linear response of TKN to PGR$_{CP}$ of this hybrid resulted similar to those of other species such as soybean (Vega et al., 2001a), oilseed rape (Dreccer et al., 2000) and wheat (Dreccer et al., 2000), and was documented in some maize hybrids when TKN and PGR$_{CP}$ were obtained as the mean of several plants within a plot (Kiniry et al., 2002), but never using individual plant values.

To study the different traits related to kernel setting of tested hybrids, we identified the numerical components of TKN: KNE$_1$, prolificacy, and KNE$_2$. Differences among hybrids in TKN were mainly associated with KNE$_1$. The highest KNE$_1$ of the newest hybrid (DK-7210) was recorded at all tested densities (Supplemental Table 2). Differences in KNE$_1$ among hybrids could be due to their different PGR$_{CP}$ (Luque et al., 2006), biomass partitioning to the apical ear (E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$) and/or the efficiency for kernel setting of this organ (KNE$_1$ E$_1$GR$_{CP}$$^{-1}$) (Echarte et al., 2004). According with previous researches, (Tollenaar et al., 1992; Andrade et al., 1999; Maddonni and Otegui, 2004) tested hybrids exhibited slightly differences in PGR$_{CP}$ and a similar response of this trait to plant density (i.e., no significant hybrid × density interaction was detected for PGR$_{CP}$). Hence, the higher KNE$_1$ of newer hybrids was not related to a higher PGR$_{CP}$. Probably, tested hybrids have a similar plant architecture (i.e., the ideotype), that has long attracted the attention of breeders to optimize light capture and plant growth under crop conditions (Lee and Tollenaar, 2007; Ku et al., 2010).

Based on the model proposed by Vega et al. (2001b), we have analyzed biomass partitioning to the ears around silking, but we have identified separately the growth of the apical ear from that of the sub-apical ear. All hybrids presented a linear response of E$_1$GR$_{CP}$ to PGR$_{CP}$ which differed from the curvilinear model proposed by Vega et al. (2001b). Hence, for the explored wide range of PGR$_{CP}$, E$_1$GR$_{CP}$ was source-limited. Comparison of E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$ among tested hybrids revealed that DK-752 (released in 1993) had the highest E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$ (ca. 0.56) while the oldest hybrid (DK-3F22) presented the lowest value (ca. 0.44). Hence, for tested hybrids, changes of KNE$_1$ were not associated with a different biomass partitioning to the apical ear but were mainly related to KNE$_1$ E$_1$GR$_{CP}$$^{-1}$. These results differed from those previously reported for an older breeding period (1965–1993) in Argentina where the higher KNE$_1$ of newer hybrids was attributed to their higher E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$ (from ca. 0.15 to 0.20) (Echarte et al., 2004).

Similarly to that described for KNE$_1$, differences in KNE$_2$ among hybrids could be attributed to E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ and/or KNE$_2$ E$_2$GR$_{CP}$$^{-1}$. The bi-linear model fitted to E$_2$GR$_{CP}$ vs PGR$_{CP}$ allowed the detection of PGR$_{CPT}$ values (3.1–4.6 g d^{-1}) above which plants of all hybrids increased biomass partitioning to sub-apical ear. This response was never documented, and the estimated PGR$_{CPT}$ values were not related to a morphogenetic limitation for the growth of the apical ear; i.e., the saturation response of E$_1$GR$_{CP}$ to PGR$_{CP}$ reported by Andrade et al. (1999) was not recorded in our data set. Plants with PGR$_{CP}$ > 3.1–4.6 g d^{-1} exhibited a positive response of E$_1$GR$_{CP}$ to PGR$_{CP}$ (Fig. 4) but did not respond setting more kernels at the apical ear (Fig. 3). For these plants, with high carbon supply around flowering, the number of florets of the apical ear would be the limiting factor of KNE$_1$ (i.e., a sink limitation; Otegui, 1997) a mechanism that was confirmed by Sarquís et al. (1998) with the addition of external supply of carbohydrates to isolated maize plants. More kernels per plant (i.e., higher TKN), however, could be obtained by the expression of fertile sub-apical ears (Sarquís et al., 1998). For PGR$_{CP}$ <3.1–4.6 g d^{-1}, the growth of sub-apical ears was drastically source limited, and plants of the newest hybrid mostly set kernels in E$_2$ when PGR$_{CP}$ > 6 g d^{-1}, as was reported for older hybrids released in Canada (Tollenaar et al., 1992) and in Argentina (Andrade et al., 1999). Additionally, the oldest hybrid set kernels in E$_2$ at lower PGR$_{CP}$ than the newest hybrid. These patterns determined that, plants of all tested hybrids for a wide range of PGR$_{CP}$ (1–4/6 g d^{-1}), mainly modified TKN by setting kernels at the apical ear, but at the lowest density some plants exceeded the PGR$_{CP}$ > 4–6 g d^{-1} and also set kernels at the sub-apical ear.

Hence, at low density (PGR$_{CP}$ > 4–6 g d^{-1}) the contribution of KNE$_2$ to TKN could be increased by a higher (i) KNE$_2$ E$_2$GR$_{CP}$$^{-1}$ and/or (ii) E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$. The first trait did not differ among hybrids and at the lowest density it was slightly lower than KNE$_1$ E$_1$GR$_{CP}$$^{-1}$ (i.e., the efficiency for kernel setting of both ears was not greatly different). By contrast, genotypic variability was recorded on E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ mainly in Exp$_1$. We speculate that breeders could consider parameters of the bilinear model fitted to E$_2$GR$_{CP}$ vs PGR$_{CP}$ (Fig. 4 and Table 5) to increase TKN. A lower PGR$_{CPT}$ (3.1 g d^{-1} vs 4.6 g d^{-1}) would impact on TKN by a higher E$_2$GR$_{CP}$. Hence, more plants of the crop would exhibit fertile sub-apical ears (i.e., higher prolificacy). By the other hand, a higher second slope of the E$_2$GR$_{CP}$ vs PGR$_{CP}$ relationship (0.60 vs 0.31) would determine a similar prolificacy but a greater KNE$_2$. To the extent of our knowledge, no information exists of the trade-off among traits related to biomass partitioning to reproductive structures and reproductive efficiency of these organs. We have explored these relationships of our data set. This analysis revealed weak trade-off between E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$ and E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ ($r = -0.47$), KNE$_1$ E$_1$GR$_{CP}$$^{-1}$ and KNE$_2$ E$_2$GR$_{CP}$$^{-1}$ ($r = -0.12$) and KNE$_1$ E$_1$GR$_{CP}$$^{-1}$ and E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$ ($r = -0.06$). By contrast, positive and strong correlations were found between KNE$_2$ E$_2$GR$_{CP}$$^{-1}$ and E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ ($r = 0.75$), prolificacy and E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ ($r = 0.79$), and prolificacy and KNE$_2$ E$_2$GR$_{CP}$$^{-1}$ ($r = 0.89$). These promising phenotypic correlations, suggest that the improvement of determinant traits of KNE$_2$ would not affect those traits related to KNE$_1$. Additionally, the selection of cultivars based on prolificacy (an easy selection trait) could also drag higher E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ and KNE$_2$ E$_2$GR$_{CP}$$^{-1}$, all traits with positive impact on KNE$_2$ and TKN, conferring a high stability of prolific hybrids under contrasting growing conditions (Adriaanse and Human, 1992; Sarquís et al., 1998; Varga et al., 2004).

5. Conclusions

We have studied the determinant traits of KNE$_1$ and KNE$_2$ of four Argentinean maize hybrids released during the last decades. The analysis was performed on a per-plant level using non-destructive techniques to estimate PGR$_{CP}$, E$_1$GR$_{CP}$, E$_2$GR$_{CP}$ and their relations with kernel setting. Within the wide range of PGR$_{CP}$ (1–10 g d^{-1}) promoted by the different densities and growing seasons, E$_1$GR$_{CP}$ and E$_2$GR$_{CP}$ were source-limited but a higher proportion of PGR$_{CP}$ was partitioned to the apical (ca. 0.39–0.57) than to the sub-apical ear (ca. 0–0.26). Moreover, E$_2$GR$_{CP}$ was almost suppressed when PGR$_{CP}$ < 3.1–4.6 g d^{-1}. At low density, the KNE$_1$ E$_1$GR$_{CP}$$^{-1}$ was slightly higher than KNE$_2$ E$_2$GR$_{CP}$$^{-1}$; hence KNE$_1$ was higher than KNE$_2$ mainly by the higher E$_1$GR$_{CP}$ PGR$_{CP}$$^{-1}$. At mid and high density, the reduced E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$and the low KNE$_2$ E$_2$GR$_{CP}$$^{-1}$ determined the drastic reduction of KNE$_2$. Differences among tested hybrids in KNE$_1$ were mainly determined by KNE$_1$ E$_1$GR$_{CP}$$^{-1}$. By contrast, hybrids had similar KNE$_2$ due to their similar E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ and KNE$_2$ E$_2$GR$_{CP}$$^{-1}$. These results suggest that breeding effect on TKN was mainly determined by KNE$_1$ E$_1$GR$_{CP}$$^{-1}$ and for the newest hybrid this trait did not interact with plant density. Hence, the newest hybrid attained the highest TKN among hybrids at low, mid and high plant density. At low density, however, contribution of KNE$_2$ to TKN could be increased if breeders would consider the selection of cultivars with higher E$_2$GR$_{CP}$ PGR$_{CP}$$^{-1}$ a trait positively related to prolificacy. This selection would be performed at low plant density. This plant density, however, is not used under current breeding practices, because breeding is focused on increasing maize yield by maintaining KNE$_1$ at high population densities (i.e., increased stress tolerance).

Acknowledgments

The authors wish to thank V. Giménez, J. Fuentes and M. Rodriguez for their valuable assistance during the experiments and Monsanto Argentina S.A. for seed supply. This work was supported by the University of Buenos Aires (UBACyT 2014-20020130100493BA), the National Agency for the promotion of Science and Technology (PICT 2012–1260) and Universidad Nacional de Lomas de Zamora. N. Ciancio and S.J.P. Incognito had a graduate's scholarship from the National Council of Research (CONICET) of Argentina. Martín Parco held a graduate's scholarship from the University of Buenos Aires. G.A. Maddonni is a member of CONICET.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2016.02.021.

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