Field Crops Research 133 (2012) 90–100

Contents lists available at SciVerse ScienceDirect

Field Crops Research

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

Inter-plant variability in maize crops grown under contrasting N × stand density combinations: Links between development, growth and kernel set

M.A. Rossini*, G.A. Maddonni, M.E. Otegui

Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA), Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires. Av. San Martín 4453 (C1417DSE), Argentina

ARTICLE INFO

Article history:
Received 14 December 2011
Received in revised form 14 March 2012
Accepted 15 March 2012

Keywords:
Maize
Zea mays L.
Plant growth
Ear development
Reproductive efficiency
N stress

ABSTRACT

Genotypic differences in the response of maize kernel number per plant to ear growth rate around silking, caused by contrasting N availability, have been attributed to the effects of this element on reproductive efficiency (i.e. kernel set per unit of ear growth rate). The objective of current research was to assess if reduced reproductive efficiency of some genotypes under N stress is due to the effect of this nutrient on the number of completely developed florets per ear, the number of exposed silks per ear, and/or abortion of pollinated florets. Two field experiments were conducted with two hybrids previously characterized by their contrasting reproductive efficiency (high for AX820 and low for AX877) under N stress, two stand densities (9 and 12 $\mathrm{pl\,m^{-2}}$) and two levels of added N (0 and 200 $\mathrm{kg\,N\,ha^{-1}}$). We established links among plant and ear growth rates, reproductive traits and kernel number per plant. Reduced reproductive efficiency (quantified as kernel number per plant per unit of spikelet growth rate around silking) of both hybrids under N deficiency was mainly due to an enhanced abortion of pollinated florets of the most suppressed plants of the stand (*dominated* individuals). This response did not appear to be the result of low spikelet growth rate around silking, but a direct control of N on sink capacity of fertilized ovaries for assimilates allocation.

1. Introduction

High stand densities used in most current maize production systems of temperate environments enhance intra-specific competition due to the early increase in growth variability among plants (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007). This trend has been reported also for maize crops grown under N deficiency (Boomsma et al., 2009), for which fertilization at the start of stem elongation reduced this variation in subsequent stages (Rossini et al., 2011). The response pattern was genotype-dependent, because recovery of crop growth and reduction of inter-plant variability were less pronounced in one hybrid (AX877) than in another one (AX820) (Rossini et al., 2011). Early differences in plant growth within the stand were sustained until the critical period for kernel set around flowering (Maddonni and Otegui, 2004), and affected biomass partitioning to the ear differently among plants (Pagano and Maddonni, 2007; Borrás et al., 2007).

D'Andrea et al. (2008) demonstrated that, under contrasting N supplies, genotypic differences in the response of kernel number per plant to plant growth rate during the critical period were related to the effects of N on biomass partitioning to the ear. This trend was not supported by Rossini et al. (2011), who found a tight relationship between ear growth rate and plant growth rate during the critical period, with no evidence of an N effect on biomass partitioning. There were, however, genotypic effects in the relationship between kernel number per plant and ear growth rate during the critical period. For AX820 this relationship was independent of factors that caused the variation in ear growth rate (e.g. stand density, N), and a single model accommodated the whole data set adequately. For AX877 the relationship was N-dependent, and two models were necessary for the correct fit of its data set. For this hybrid, reduced N availability caused a decrease in reproductive efficiency expressed as kernel number per unit of ear growth rate during the critical period.

Negative effects of stress on biomass partitioning to harvestable reproductive organs during the critical period for kernel set have been reported for different species in a previous research (Vega et al., 2001). This study highlighted that maize plants subjected to reduced irradiance per plant at high stand density experienced a larger decrease in the reproductive/vegetative ratio than sunflower or soybean plants. A clear sign of reduced assimilate allocation to the ear is a longer delay in silking date than in anthesis date with the concomitant increase in the anthesis-silking interval (ASI). This response has been broadly documented at the plant population level (i.e. based on 50% anthesis and 50% silking dates of the stand), particularly for water (Hall et al., 1982; Bolaños and Edmeades,

---

* Corresponding author. Tel.: +54 11 45248039; fax: +54 11 45148739.
E-mail address: mrossini@agro.uba.ar (M.A. Rossini).

0378-4290/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.fcr.2012.03.010

1993), N (Jacobs and Pearson, 1991; D'Andrea et al., 2009) and stand density (Edmeades and Daynard, 1979; Sangoi et al., 2002) effects. Therefore, the ASI is generally used as a secondary trait in breeding programs targeting stress-prone environments (Bänziger and Laffite, 1997), particularly because of its simple representation of canopy performance and partitioning to the ear. At the individual plant level, however, the ASI has some limitations for the correct interpretation of the underlying physiological processes responsible of the success or failure for setting a kernel by a fertilized ovary (Uribelarrea et al., 2002). Consequently, other traits at the plant level may give a better explanation to the observed reduction in maize reproductive efficiency in response to N stress, like floret development at silking (i.e. morphogenetic limitations to kernel set), synchrony in silk exposure among florets of the same ear (i.e. pollination timing or pollination failure limitations to kernel set), and/or abortion of fertilized ovaries (i.e. metabolic limitations to kernel set).

Previous studies have shown that the number of completely developed florets per ear is not substantially affected by variations in assimilate provision to this organ caused by stand densities (Otegui, 1997; Cárcova et al., 2000), sowing dates (Cirilo and Andrade, 1994; Otegui and Melón, 1997), nutrient offer (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995b; Monneveux et al., 2005), water availability (Edmeades et al., 1993; Otegui et al., 1995), or above optimum temperatures (Rattalino Edreira et al., 2011). Reports on the pattern of silk exposure from individual ears (Cárcova et al., 2000; Uribelarrea et al., 2002; Lizaso et al., 2003) and the subsequent progress of fertilized ovaries (Otegui et al., 1995; Cárcova et al., 2000; Lizaso et al., 2003; Cárcova and Otegui, 2007) in field conditions are comparatively rare, particularly under stress conditions (Otegui et al., 1995; Rattalino Edreira et al., 2011). Moreover, the studies cited did not analyze the variability of these traits at the plant population level.

In a field study where plants were identified from the onset of the heterotrophic stage onwards (V_3; Ritchie and Hanway, 1982), the number of completely developed florets per ear produced by *dominated* individuals at high stand density did not differ from the number produced by the *dominant* ones (Pagano et al., 2007). Nevertheless, both categories did differ in the rate of progress of floret development within the ear, which was slower in *dominated* than in *dominant* individuals. Consequently, ASI values were larger for the former than for the latter. Additionally, synchrony in silk exposure was reduced in *dominated* plants (i.e. the time lag between early- and late-appearing silks from an ear increased in these plants), due to reduced spikelet growth rate during the critical period. Borrás et al. (2007) determined that the capacity of a plant to reach silking depended upon its capacity to reach a minimum ear biomass during the critical period for kernel set. Individuals with ears below this threshold were barren. Moreover, barrenness was also registered among plants that exposed their silks. In this case, barrenness may be attributed to pollination failure due to lack of pollen at their time of silking (Hall et al., 1981; Bassetti and Westgate, 1993; Uribelarrea et al., 2002). When adequate pollen availability was granted by means of a late-pollinator source, the only explanation for their barrenness was kernel abortion (Westgate and Boyer, 1986; Otegui et al., 1995) caused by growth inhibition of early-pollinated ovaries on the late-pollinated ones (Cárcova and Otegui, 2001, 2007). Therefore, any restriction to plant growth (e.g. by reduced light, water or nutrient availability) around flowering may exert a negative effect on kernel set, partially due to impaired silk exposure (Borrás et al., 2009) but also attributable to reduced pollination synchrony. This lack of synchrony may cause a reduction in the number of ovaries that are fertilized within a critical window of 2–4 days after individual plant silking, which increases kernel abortion (Cárcova and Otegui, 2001, 2007). The inability to reverse abortion caused by N deficiency in fertilized ovaries infused with sucrose, and the partial reduction of abortion when they were infused with N (Below et al., 2000) emphasized the direct role of this nutrient on the ability to use carbon from the grains. This result contrasts with data found by Boyle et al. (1991) and Zinselmeier et al. (1995), who partially reversed reproductive failure induced by water stress by means of sucrose infusion to plants.

Information is available on the general flowering pattern (i.e. anthesis and silking) of *dominant* and *dominated* plants (Borrás et al., 2009). However, there is (i) only one reference linking these traits to early reproductive development and its effects on final kernel number per plant (Pagano et al., 2007), and (ii) no reference linking these traits to growth conditions responsible of the early establishment of contrasting plant categories within the stand; i.e. how the limiting factor type (aerial or soil resource) affects the symmetry among plants for its acquisition (Rossini et al., 2011; Caviglia and Melchiori, 2011). The analysis of population variability in reproductive development may improve our understanding of processes controlling the variation in reproductive efficiency among genotypes, which has been recently document for two maize hybrids grown under contrasting N levels (Rossini et al., 2011). Our objective in current research was to analyze if reduced reproductive efficiency of AX877 under N deficiency is due to N effects on (i) the number of completely developed florets per ear, (ii) the number of silks exposed per ear, and/or (iii) kernel abortion. We hypothesize that the reduced reproductive efficiency of AX877 under N stress is due to an enhanced abortion of pollinated florets, predominantly among the most suppressed plants of the stand (*dominated* individuals).

2. Materials and methods

2.1. Crop husbandry, treatments and experimental design

Field experiments were conducted during 2006–2007 (Exp.1) and 2007–2008 (Exp.2) at the Pergamino station (33°56′S, 60°34′W) of the National Institute for Agricultural Technology (INTA) on a silty clay loam soil (typic Argiudoll). The uppermost soil profile (0–40 cm) had levels of 23 g kg^{-1} for organic matter, 115 mg kg^{-1} for mineral P, and 14 g kg^{-1} for N-NO$_3$. Treatments included a factorial combination of two single-cross maize hybrids from Nidera Argentina, two stand densities and two N levels. Hybrids were selected for their contrasting reproductive efficiency under N stress by Rossini et al. (2011). These hybrids were the AX820 CL-MG (hereafter AX820) with a high reproductive efficiency, and the AX877 CL-MG (hereafter AX877) with a low reproductive efficiency. Hybrids shared a common female inbred of the Lancaster heterotic group (D. Novoa, Nidera Argentina, personal communication). Hybrid AX820 was released during 2004 and AX877 during 2005. Tested stand densities were 9 (D$_9$) and 12 (D$_{12}$) plants m^{-2}. N levels were a control with no added N (N$_0$), and a fertilized condition with 200 kg of N ha^{-1} (N$_{200}$) added as urea at V_6, formerly identified as the stage when variability in plant growth among individuals of the stand is stabilized (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007). The N$_0$ treatment was considered a N-stressed condition for modern maize hybrids grown on soils with 23 g kg^{-1} organic matter, as demonstrated in previous work (D'Andrea et al., 2008).

Treatments were distributed in a split-plot design, with N levels in the main plots and all hybrid × stand density combinations in the sub-plots (hereafter termed plots). Plots had six rows, 0.7 m between rows, and 18 m length. Sowing was performed manually on 20-Oct (Exp.1) or 22-Oct (Exp.2), at a rate of 3–4 seeds per hill and thinned to one plant per site at the end of the heterotrophic phase (V_3; Pommel, 1990). All experiments were kept free of weeds by means of chemical controls (4 L ha^{-1} of atrazine at 0.5 a.i. + 2 L ha^{-1} of acetochlor at 0.98 a.i.) and hand weeding. Water deficit was prevented by means of sprinkler irrigation, with the uppermost 1 m of soil profile held near field capacity through the whole cycle. Irrigation schedule (timing and water amount) was determined from daily records of rainfalls and estimated crop evapo-transpiration values. As total rainfalls from October 2006 to March 2007 were larger (1000 mm) than during the same period of Exp.2 (500 mm), the amounts of water irrigation were 75 mm in Exp.1 and 240 mm in Exp.2. All treatments of each experiment were irrigated with the same amount of water. Meteorological conditions were registered in a weather station located at 500 m from the experiment, and thermal time was computed from sowing onwards (base temperature of 8 °C; Ritchie and NeSmith, 1991) based on daily mean air temperatures using hourly-registered data.

2.2. Plant and ear growth rates

At V_3, 10 (Exp.1) or 12 (Exp.2) consecutive plants of similar size (visual assessment) were tagged at each plot. Vegetative (V_n) and reproductive (R_n) stages were recorded weekly (V stages) or daily (R stages) for each tagged plant (Ritchie and Hanway, 1982), including anthesis date (at least one anther visible in the tassel) and silking date (at least one silk visible in the apical ear). Biomass of all tagged plants was estimated weekly between V_3 and R_2 by means of models described in a previous work (Rossini et al., 2011) and widely used in this species (Andrade et al., 1999; Vega et al., 2001; Echarte and Tollenaar, 2006; D'Andrea et al., 2008). Briefly, destructive plant samplings (n = 20–30, per hybrid) were performed weekly to derive allometric relationships between biomass and morphometric variables of plant (height from ground level to the ligule of the uppermost ligulated leaf, and basal stem diameter) and apical ear (maximum diameter size). On each sampling date, morphometric variables were also registered on tagged plants, and the established allometric models were used for estimating their biomass (i.e. whole plant shoot between V_3 and R_2, and apical ear shoot at R_1 and R_2). Tagged plants were individually sampled at physiological maturity (R_6), and shoot biomass, grain yield (in g pl^{-1}) and kernel number per plant were registered for each of them. Individual kernel weight (in mg) was computed as the quotient between grain yield and kernel number per plant.

Plant (in g d^{-1}) and ear (in g d^{-1}) growth rates of each tagged plant were estimated as the slope of the relationship between cumulative plant or ear biomass and time. Plant growth rate was obtained for the early reproductive period that spans between V_7 and V_{12}, and the critical period that spans between silking – 227 °C d (ca. V_{12}) and R_2 (Otegui and Bonhomme, 1998). Ear growth rate was computed for the critical period and was based on (i) an ear biomass value of zero at 227 °C d before R_1, (ii) estimated ear biomass at R_1, and (iii) estimated ear biomass at R_2.

The spikelet growth rate during the critical period (in mg d^{-1}) was estimated as the quotient between ear growth rate during the critical period and the maximum number of completely developed florets per ear (described next). The reproductive efficiency (in kernels d mg^{-1}) was computed as the quotient between kernel number per plant and spikelet growth rate during the critical period.

2.3. Ear development

Ear development was analyzed for the period between $V_6 - V_7$ and R_1. For this purpose, six sites of 10 (Exp.1) or 12 (Exp.2) consecutive plants were identified at V_3 in each plot. At weekly intervals, all plants in a site were harvested and morphometric variables (plant height, stalk diameter at the base of the stem) measured in each plant for the allometric estimation of plant biomass (Rossini et al., 2011). The apical ear of each harvested plant was separated from the rest and used for computing (i) the number of spikelet rows per ear, measured in its mid portion, and (ii) the number of spikelets per row and the degree of floret development of each spikelet along a row, both measured on two opposite rows. These observations were made with a Leica Mz6 stereomicroscope (6.3–40×, McBrain Instruments, Switzerland), and data classified using a qualitative scale adapted from Otegui and Melón (1997).

A sigmoid model (Eq. (1)) was fitted to data representative of the cumulative number of completely developed florets (i.e. those with a silk > 1 mm) along thermal time.

$$\mathrm{NFPE} = \frac{a_1 + b_1}{1 + e^{-(\mathrm{TT}-c_1)/d_1}} \qquad (1)$$

where NFPE is the number of completely developed florets per ear; $a_1 + b_1$ is the maximum value of NFPE; TT is thermal time from sowing and c_1 is the TT to 50% of maximum number of completely developed florets, $(a_1 + b_1)/2$ is the NFPE value at the inflection point of the function, and $1/(b_1 d_1)$ is a proportionality function.

2.4. Flowering dynamic and silk emergence

Anthesis and silking dates were registered for each plant tagged for biomass estimation (Section 2.2). Data were collected daily from the start of flowering (i.e. first plant with a visible event) onwards. A sigmoid model (Eq. (2)) of the type describe in Eq. (1) was fitted to the evolution of the cumulative value of the proportion of plants that reached anthesis and silking, and model parameters were used for the analysis of flowering dynamics at the plant population level (Hall et al., 1980, 1981; Maddonni et al., 1999).

$$\mathrm{Proportion} = \frac{a_2 + b_2}{1 + e^{-(\mathrm{TT}-c_2)/d_2}} \qquad (2)$$

The ASI of each plot was computed in two ways (Uribelarrea et al., 2002), at the plant population level and at the individual plant level. The former was obtained as the difference in c_2 parameters of functions fitted to anthesis and silking data. The latter was computed as the average of ASI values obtained from all tagged plants (i.e. difference between observed anthesis and silking dates of each plant).

Silk emergence was quantified on 10 (Exp.1) or 12 (Exp.2) plants per plot, different from those used for biomass estimation (Section 2.2) or floret development (Section 2.3). Silking date (day 1) was registered for each plant, and apical ears were collected on day 5. Starting from the base of the ear, three categories of florets were identified along two opposite spikelet rows: (i) florets with silks exposed from the husks, (ii) florets with silks > 1 mm (florets in stage E; Otegui and Melón, 1997) but not exposed from the husks, and (iii) florets with silks < 1 mm. The number of completely developed florets per ear was also computed in these ears as the product between the number of florets with silks > 1 mm (average of two spikelet rows) and the number of spikelet rows per ear (observed at the mid portion of the ear). The number of silks exposed from the apical ear was estimated as the product between the number of silks exposed per spikelet row and the total number of spikelet rows per ear.

2.5. Sources of loss from potential kernel number per plant

We established three sources of loss in potential kernel number per plant caused by N stress, stand density stress and plant category. The first source (Loss 1) represented the reduction caused by a decrease in the number of completely developed florets per ear (i.e. morphogenetic restriction at the axillary meristem level). This loss was considered null for *dominant* plants of the D_9N_{200} treatment of each hybrid, which was set as control value. It was computed as in Eq. (3) for each other treatment combination.

$$\text{Loss}\quad 1 = 1 - \left(\frac{\text{NFPE}_\text{T}}{\text{NFPE}_\text{C}}\right) \tag{3}$$

where NFPE_T and NFPE_C are the number of completely developed florets per ear of a treatment and the control, respectively.

For the computation of Loss 1, measurements were performed on ears used for the determination of silk emergence (Section 2.4). Losses 2 and 3 were computed for *dominant* and *dominated* individuals in each N × stand density combination. Loss 2 (Eq. (4)) represented the proportion of the number of completely developed florets per ear that did not expose silks, and loss 3 (Eq. (5)) the proportion of pollinated silks that did not set kernels (i.e. kernel abortion).

$$\text{Loss}\quad 2 = 1 - \left(\frac{\text{Number of silks exposed per ear}}{\text{Number of completely developed florets per ear}}\right) \tag{4}$$

$$\text{Loss}\quad 3 = 1 - \left(\frac{\text{Kernel number per plant}}{\text{Number of silks exposed per ear}}\right) \tag{5}$$

2.6. *Method for plant classification in dominant and dominated groups*

Total plant biomass at physiological maturity was taken as indicator of plant competitive capacity for resource capture within the stand. Based on this criterion, each plant was classified as *dominant* or *dominated* when its biomass corresponded to the uppermost or lowermost 33% of the data set for this trait in each treatment combination, respectively (Maddonni and Otegui, 2004). Other measured traits (plant growth rate during early reproductive period, ear growth rate during the critical period, anthesis and silking dates, ASI and kernel number per plant) were linked to this classification, except for the developmental stages of the ear, and the proportion of non exposed silks. For these two traits, plants were assigned to the *dominant* or *dominated* category based on plant biomass at (i) the time of sampling (Section 2.3) for ear development, and (ii) R_1 for the proportion of unexposed silks (Pagano et al., 2007).

2.7. *Data analysis*

Mean values of each trait were obtained for (i) all tagged plants (mean plot value), and (ii) each extreme plant category (i.e. *dominant* and *dominated* individuals). For the former, the coefficient of variation (CV) was computed and used for the characterization of the variability of each trait among plants. 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 computed 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.

Treatment effects on mean values (per plot and per plant category) were evaluated by ANOVA. The ANOVA was performed combining the experiments over locations, with the experiment as a random variable and N, plant density as fix variables. For plant categories (fix variable), this condition was included in the ANOVA as a sub-factor within each N × stand density × hybrid combination. The relationship between variables was evaluated by means of regression analysis, and models fitted using TBLCURVE (Jandel, 1992). Significant differences between fitted models across treatments were established by ANOVA of model parameters. Linear functions were used for the analysis of most relationships, except for the response of (i) ASI of individual plants to spikelet growth rate during the critical period (exponential function), and (ii) Losses 2 and 3 to spikelet growth rate during the critical period (power function). Several functions were tested and those selected had biological sense and the highest r^2. For each hybrid, an exponential function was used for the estimation of a boundary function of maximum reproductive efficiency in response to spikelet growth rate during the critical period. Data pairs included in the analysis were selected based on the methodology proposed by Otegui and Bonhomme (1998). Briefly, after sorting data in a descending order of spikelet growth rate during the critical period, only data pairs with increasing values of reproductive efficiency were kept for calculation. Residual values to the boundary function (i.e. observed minus estimated reproductive efficiency) were calculated for each data, and treatment effects on mean residual values were tested by ANOVA.

3. Results

3.1. *Anthesis and silking dynamics at the plant population level and interplant variability of both traits*

Thermal time to anthesis (parameter c_2) was shorter ($P < 0.001$) for hybrid AX820 than for hybrid AX877 (Table 1). For all hybrid × stand density combinations, N deficiency caused a delay of ca. 50 °C d in the thermal time to anthesis ($P < 0.05$) and a decline ($P < 0.05$) in the rate of progress to this event (i.e. enhanced value of parameter d_2). Hybrid AX877 had a longer ($P < 0.001$) thermal time to silking than the AX820 (Table 1). For both hybrids, N deficiency produced a delay in this trait during Exp.2, but this trend held only for AX877 in Exp.1 ($P < 0.05$ for Experiment × N × hybrid; data not shown). Hybrid AX820 had a shorter ($P < 0.001$) ASI than the AX877 (Table 1), and N deficiency enhanced the ASI only for the latter in Exp.1 ($P < 0.05$ for Experiment × N × hybrid; data not shown). For all hybrid × N level combinations, the ASI was ∼9 °C d longer ($P < 0.05$) in D_{12} than in D_9 (Table 1).

Hybrid AX877 had a larger ($P < 0.05$) plant population variability in anthesis and silking dates than hybrid AX820 (Table 1). N deficiency caused an increase in the CV of anthesis date in both experiments ($P < 0.05$) and of silking date in Exp.1 ($P < 0.01$ for Experiment × N; data not shown), whereas enhanced stand density caused an increase in the CV of silking date during Exp.2 ($P < 0.05$ for Experiment × stand density; data not shown). The CV of the ASI of individual plants did not differ between hybrids and was not affected by N deficiency or stand density (Table 1).

3.2. *Number of florets, exposed silks and kernels in the apical ear. Sources of loss between potential and actual kernel numbers*

For both hybrids, kernel number per plant decreased in response to N deficiency and enhanced crowding ($P < 0.01$). The number of completely developed florets and the number of silks exposed per ear decreased only in response to reduced N level ($P < 0.001$; Table 2), and both traits were larger for AX877 than for AX820 ($P < 0.001$). Hybrids, however, did not differ in the number of kernels per plant. As compared to the optimum growing condition (D_9N_{200}), N deficiency caused a 10% decrease ($P < 0.001$) in the number of completely developed florets per ear (Loss 1), but enhanced crowding had no effect on this trait (Table 2). Stressful growing conditions (i.e. N_0 and D_{12}) promoted a reduction ($0.001 < P < 0.05$) in the number of silks exposed per ear (Loss 2) and an increased ($P < 0.05$) abortion of fertilized ovaries (Loss 3). N deficiency effects on Losses 2 and 3 were larger than those computed for enhanced crowding (Table 2), and loss in kernel numbers due to kernel abortion (Loss 3) was larger than that attributable to pollination failure (Loss 2).

Table 1
Mean values and ANOVA of parameters from sigmoid functions fitted to anthesis and silking dynamics, anthesis-silking interval at plant population level (ASI_{PP}) and coefficient of variation (CV) of days to anthesis, days to silking and ASI of individual plants (ASI_{IP}). Values are the mean of main factors.

Main factor		Anthesis				Silking				ASI_{PP}	CV %		
		a_2	b_2	c_2	d_2	a_2	b_2	c_2	d_2	°C d	Anthesis	Silking	ASI_{IP}
Experiment	1	0.50	0.50	887	9.97	0.50	0.50	907	10.94	20.08	45	49	23
	2	0.50	0.50	883	14.90	0.50	0.50	913	19.44	30.44	51	59	29
P		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N	0	0.50	0.50	910	13.08	0.5	0.5	939	16.75	29.39	50	55	24
(kg ha^{-1})	200	0.50	0.50	860	11.80	0.5	0.5	881	13.62	22.38	47	54	29
P		ns	ns	*	*	ns	ns	*	ns	ns	*	ns	ns
Hybrid	AX820	0.50	0.50	850	11.85	0.50	0.50	866	14.32	17.53	45	50	25
	AX877	0.49	0.49	920	13.03	0.50	0.50	954	16.06	34.24	52	59	27
P		ns	ns	***	ns	ns	ns	***	ns	***	*	*	ns
Density	9	0.50	0.50	883	11.57	0.50	0.50	903	13.38	21.45	50	54	23
(pl m^{-2})	12	0.50	0.50	887	13.31	0.50	0.50	917	17.00	30.32	47	56	29
P		ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
The significance level of the interactions is not presented.

3.3. Floret development in apical ears of dominant and dominated plants

N deficiency reduced the number of florets per ear row ($P < 0.05$), and this trend started at 475 °C d (ca. V_7) in Exp.1, and at 870 °C d (ca. V_{16}) in Exp.2 (data not shown). Increased stand density did not modify this trait. For each treatment combination, the number of florets per ear row was smaller ($0.001 < P < 0.05$) in *dominated* plants than in *dominant* individuals. Early in the cycle (ca. 550 °C d from sowing), hybrid AX820 had more differentiated florets than hybrid AX877 ($0.001 < P < 0.05$). Final floret number, however, was larger for the latter (ca. 734 florets per ear) than for the former (ca. 674 florets per ear).

In both experiments, reduced N level caused a decrease ($0.01 < P < 0.05$) in the number of completely developed florets (i.e. E stage) from the onset of their detection at ca. 730 °C d. Similarly, *dominant* individuals always had a larger number of florets at the E stage than the *dominated* ones (i.e. $a_1 + b_1$ in Table 3; $P < 0.001$).

N deficiency caused an increase in the required thermal time for reaching the condition of ears having 50% of their florets in the E stage (parameter c_1; $P < 0.05$; Table 3). In both experiments, this requirement was larger for hybrid AX877 than for hybrid AX820 ($P < 0.001$, Table 3). The former also had a larger final number of florets in this stage than the latter ($P < 0.001$). In Exp.1, stand density affected the parameter c_1 of hybrid AX877, which reached values of ca. 797 °C d in D_9 and ca. 848 °C d in D_{12} ($P < 0.05$ for Experiment × hybrid × stand density; data not shown).

Parameters a_1, b_1 and c_1 differed between plant categories in both experiments ($P < 0.001$, Table 3). The *dominant* individuals had a larger number of completely developed florets than the *dominated* ones at both stand densities in Exp.2. This trend held only at D_{12} in Exp.1 ($P < 0.05$ for Experiment × stand density × plant category;

Table 2
Mean values and ANOVA of final number of completely developed florets per apical ear (NFPE), number of silks exposed from apical ear (NSEE), kernel number per plant (KNP), and three sources of loss in potential kernel numbers. Each loss represents the reduction caused by a decrease in the NFPE (Loss 1), the proportion of the NFPE that did not expose silks (Loss 2) and the proportion of NSEE that did not set kernels (Loss 3). Values are the mean of main factors.

Main factor		NFPE	NSEE	KNP	Loss 1%	Loss 2%	Loss 3%
Experiment	1	701	599	430	4.7	15.3	28.1
	2	550	550	352	6.3	20.1	36.6
P		ns	*	ns	ns	*	ns
N	0	659	534	318	9.9	19.4	40.6
(kg ha^{-1})	200	726	626	474	1.0	13.9	24.2
P		***	***	**	***	***	*
Hybrid	AX820	637	546	391	5.4	17.2	29.3
	AX877	728	614	405	5.5	16.1	35.5
P		***	***	ns	ns	ns	ns
Density	9	698	592	430	4.6	15.7	28.1
(pl m^{-2})	12	687	568	363	6.2	17.6	36.6
P		ns	ns	**	ns	*	*

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
The significance level of the interactions is not presented.

Table 3
Mean values and ANOVA of parameters from sigmoid functions fitted to the evolution of the number of completely developed florets per apical ear. Sum of parameters a_1 and b_1 represents the maximum value of completely developed florets per apical ear; c_1 is the thermal time to 50% of maximum number of completely developed florets; $(a_1 + b_1)/2$ is the number of completely developed florets per apical ear at the inflection point of the function; and $1/(b_1 d_1)$ is a proportionality function. Values are the mean of main factors.

Main factor		Parameters			
		a_1	b_1	c_1	d_1
Experiment	1	345	345	797	22.55
	2	334	334	790	17.97
P		**	**	ns	*
N	0	325	325	821	21.73
(kg ha^{-1})	200	354	354	765	18.78
P		**	**	*	ns
Hybrid	AX820	323	323	766	19.38
	AX877	356	356	820	21.14
P		***	***	***	ns
Density	9	342	342	785	19.29
(pl m^{-2})	12	337	337	801	21.23
P		ns	ns	ns	ns
Plant Category	Dominated	324	324	814	20.75
	Dominant	355	355	772	19.77
P		***	***	***	ns

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
The significance level of the interactions is not presented.

data not shown). During both experiments, thermal time requirement to 50% of florets at the E stage was longer for *dominated* than *dominant* plants only at N_0 ($P < 0.05$ for N × plant category; data not shown).

The number of kernels per ear was affected ($0.001 < P < 0.05$) by N level, stand density (Table 2) and plant category in both experiments. During Exp.1, differences between N levels were larger for AX877 than for AX820 ($P < 0.05$ for Experiment × N × hybrid; data not shown). Additionally, during Exp.2 differences in this trait were larger between plant categories of AX877 than of AX820 ($P < 0.01$ for Experiment × hybrid × plant category; data not shown). This difference was 271 kernels per ear for the former and 153 kernels per ear for the latter.

3.4. *Relationships between reproductive development and kernel set in dominant and dominated individuals*

Experiment effects on several traits (Section 3.3) did not modify the relationships among variables. Thus, functions were fitted combining results of both experiments.

Plant growth rate at the start of shoot elongation was reduced by N deficiency ($P < 0.05$), increased stand density ($P < 0.05$) and *dominated* individuals ($P < 0.001$), and the response range was larger for AX877 (between 1.11 and 4.96 g pl^{-1} d^{-1}; i.e. 78% decrease under stress) than for AX820 (between 0.93 and 3.32 g pl^{-1} d^{-1}; i.e. 72% decrease under stress). Variation in this trait explained 64% (AX820) and 58% (AX877) of observed variation in thermal time to 50% E stage ($P < 0.001$; Fig. 1a) among plant categories of the different N × stand density combinations. The range of thermal time to 50% E stage, however, did not differ between hybrids (range of 150 °C d for AX820 and of 175 °C d for AX877).

The range of thermal time to silking of the different plant types at each N × stand density combination was narrower for AX820 (between 821 and 945 °C d) than for AX877 (between 896 and 1061 °C d), and was significantly related to ear reproductive development represented by thermal time to 50% E stage ($P < 0.001$; Fig. 1b). Fitted models, however, differed between hybrids because the ordinate value was larger for AX820 than for AX877 ($P < 0.001$) and the slope of the latter was closer to 1 than that of the former. Consequently, delayed floret development promoted by N stress, plant category and stand density, caused an almost equivalent delayed in silking for the population of plants of AX877 but not for that of AX820. These differences between hybrids disappeared for the response of ASI of individual plants to thermal time to silking (Fig. 1c).

Kernel number per plant decreased linearly in response to increased ASI of individual plants (Fig. 1d), and fitted models differed in ordinate ($P < 0.001$) but not in slope values. Therefore, interesting differences were detected between hybrids. On one hand, the AX820 set fewer kernels than the AX877 at a same ASI value. On the other hand, both hybrids explored a similar range of kernel number per plant because ASI values were smaller for hybrid AX820 than for hybrid AX877. Evaluation of the different sources of kernel loss respect to potential kernel numbers indicated that the response of kernel number per plant responded weakly to reduction in (i) the number of completely developed florets per ear ($r^2 = 0.50$ for AX820 and 0.27 for AX877; $P \leq 0.05$), and (ii) the number of silks exposed per ear ($r^2 = 0.45$ for AX820 and 0.39 for AX877; $P \leq 0.01$). In both cases, negative residuals corresponded mostly to *dominated* individuals (data not shown). Model fit based on plant categories, however, was significant only for Loss 2 of hybrid AX820, for which the response observed among *dominated* plants was steeper (KNP = 715–2298 Loss 2; $r^2 = 0.90$; $P < 0.001$) than that observed among the *dominant* ones (KNP = 699–1340 Loss 2; $r^2 = 0.72$; $P < 0.01$). On the other hand, kernel number per plant was strongly related to kernel abortion represented by Loss 3, for which a single linear model accommodated the variation introduced by all N × stand density combinations and plant categories of AX820 (KNP = 572–637 Loss 3; $r^2 = 0.87$; $P < 0.001$) and AX877 (KNP = 638–666 Loss 3; $r^2 = 0.95$; $P < 0.001$). For this source of loss, N deficiency caused (i) a decreased kernel set in both plant categories of hybrid AX877 during Exp.1 ($P < 0.05$ for Experiment × N × hybrid), and (ii) a larger reduction in kernel set of *dominated* individuals of AX877 than of AX820 in Exp.2 ($P < 0.05$ for Experiment × N × hybrid × plant type). No clear trend was detected for this source of loss in other treatment combinations.

3.5. *Relationships between plant growth, biomass allocation to reproductive organs and final kernel numbers. Links with reproductive development*

Variations in plant growth rate during the critical period were well explained by variations registered in plant growth rate during the early reproductive period (Fig. 2a), with no distinction between hybrids. However, hybrids did differ ($P < 0.05$) in biomass partitioning during the critical period (relationship between ear growth rate and plant growth rate during the critical period), which was larger for AX820 than for AX877 (Fig. 2b). Due to the small variation registered in the number of completely developed florets per ear of each hybrid (Table 2), there was a very strong and simple linear relationship between spikelet growth rate and ear growth rate during the critical period (Fig. 2c), which did not differ between hybrids.

Variations in spikelet growth rate during the critical period had large effects on the ASI of individual plants, and significant differences ($P < 0.05$) were detected between hybrids in fitted models (Fig. 3a). For the explored ranges of spikelet growth rate (AX820 > AX877), the ASI of individual plants of both hybrids decreased in response to increased assimilates availability per spikelet. For a same level of this variable, plants of AX820 always attained lower values of ASI than those of AX877. Interestingly, hybrids did not differ in the response of Loss 2 (i.e. pollination failure due to reduced number of exposed silks) to spikelet growth rate during the critical period (Fig. 3b), neither in the response of kernel abortion (Loss 3) to this trait (Fig. 3c). The latter was opposite in trend to the relationship between kernel number per plant and spikelet growth rate during the critical period (Fig. 3d). These response patterns (Fig. 3b and c) explained the tight linear relationship between kernel number per plant and Loss 3 described in the last paragraph of the previous section. Links between plant growth, reproductive development and kernel number per plant were summarized in a flow chart (Fig. 4).

During Exp.1 the reproductive efficiency (derived from the relationship depicted in Fig. 3d) was larger ($P < 0.001$) for hybrid AX877 (ca. 373 kernels d mg^{-1}) than for hybrid AX820 (ca. 195 kernels d mg^{-1}). During Exp.2 the reproductive efficiency of both hybrids decreased in response to reduced N level ($P < 0.01$), especially among the *dominated* individuals of the stand (decrease under N stress of 96 and 19 kernels d mg^{-1} for *dominated* and *dominant* plants, respectively; $P < 0.05$). Additionally, extreme plant categories of hybrid AX877 differed in this trait ($P < 0.01$), which was larger for *dominant* (325 kernels d mg^{-1}) than for *dominated* (220 kernels d mg^{-1}) individuals. Therefore, a strong negative relationship was established for the boundary function between reproductive efficiency and spikelet growth rate during the critical period (Fig. 5), which was based predominantly on the response of dominant individuals of both hybrids (nine out of thirteen data pairs for AX820 and eight out of eleven data pairs for AX877). For both hybrids, *dominant* plants had a similar mean residual value across N levels (−43 and −10 kernels d mg^{-1} at N_0 and N_{200}, respectively), but *dominated* individuals had a larger mean residual value

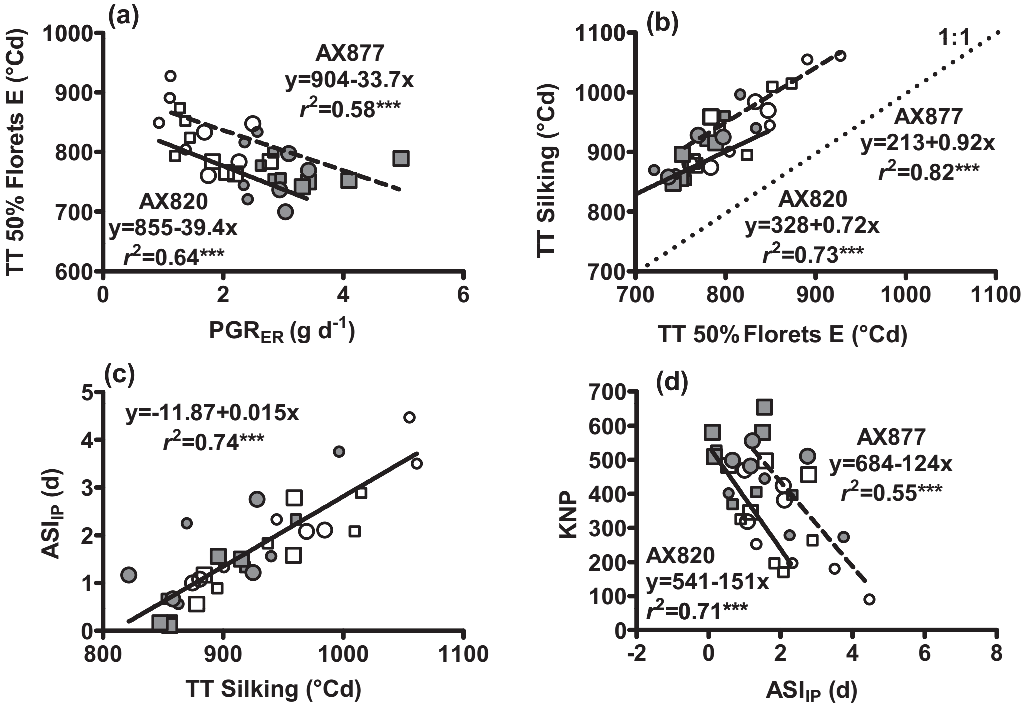

Fig. 1. Relationships between thermal time (TT) to 50% of the maximum number of completely developed florets in the apical ear (TT 50% Florets E) and plant growth rate during the early reproductive period (PGR_{ER}) (a), TT to silking and TT 50% Florets E (b), the anthesis-silking interval of individual plants (ASI_{IP}) and TT to silking (c) and kernel number per plant (KNP) and ASI_{IP} (d). Grey (N_{200}) and empty (N_0) symbols are for N levels; small (*dominated* plants) and large (*dominant* plants) symbols are for plant categories; squares (9 pl m^{-2}) and circles (12 pl m^{-2}) are for stand densities. When significant differences were detected between hybrids ($P \leq 0.05$), solid and dotted lines represent fitted models for AX820 and AX877, respectively.

at N_0 (−165 kernels d mg^{-1}) than at N_{200} (−64 kernels d mg^{-1}; $P < 0.01$ for N × plant category).

4. Discussion

N stress effects on kernel number per plant have been associated with reductions in plant growth rate during the critical period (Uhart and Andrade, 1995a; Andrade et al., 2002). Some evidences (D'Andrea et al., 2006, 2008) indicated additional negative effects of low N availability on biomass partitioning to the ear (i.e. reduced ear growth rate per unit of plant growth rate during the critical period) and on kernel set per unit of ear growth rate during critical period (i.e. reduced reproductive efficiency). A recent study (Rossini et al., 2011) added information of the negative effects of N deficiency on reproductive efficiency only for a hybrid with high intrinsic (i.e. constitutive) population variability (AX877) and not among the more uniform plants of the other tested hybrid (AX820). In the current research, we evaluated how the availability of contrasting resources (aerial for light and soil for N) affected plant reproductive development (i.e. number of completely developed florets per ear, number of silks exposed per ear, and kernel abortion) of these same hybrids, and computed different sources of loss between actual (kernel number per plant) and potential kernel numbers (florets per ear and silks exposed per ear). We evaluated if reductions in reproductive efficiency observed under N stress were linked to negative effects on plant reproductive development. We also established links between growth variables (plant growth rate during early reproductive period, plant and ear growth rates during the critical period) analyzed in previous research (Rossini et al., 2011) and mentioned reproductive characteristics (Fig. 4), as well as between kernel number per plant and spikelet growth rate during the critical period (Fig. 3d). Spikelet growth rate was priori considered a better descriptor of final ovary fate (Edmeades et al., 1993) than plant growth rate during the critical period. Therefore, we redefined reproductive efficiency as kernel number per plant per unit of spikelet growth rate during the critical period.

N deficiency and enhanced crowding caused the usual symptoms of abiotic stress on the reproductive dynamics of maize crops. We detected the expected delay in thermal time to silking and increase in ASI of the population of plants (Hall et al., 1982; Bolaños and Edmeades, 1993; D'Andrea et al., 2009), as well as an enhanced proportion of plants that did not reach silking (Hall et al., 1982; Uribelarrea et al., 2002). All these responses, simple to incorporate in breeding screenings, are evidence of the usually higher sensitivity to abiotic stress of maize female organ as compare to its male counterpart (Edmeades and Daynard, 1979; Hall et al., 1982; Jacobs and Pearson, 1991; Lizaso and Ritchie, 1997; Chapman and Edmeades, 1999; Sangoi et al., 2002), which is attributed to a reduced flux of assimilates to the ear (Boyle et al., 1991; Schussler and Westgate, 1991, 1994). Responses to stress, however, were more pronounced for hybrid AX877 than for the AX820. For both hybrids, increased variability in individual plant growth in response to N stress was detected before the critical period (evidenced in plant growth rate during early reproductive period), but it was larger among plants of AX877 than of AX820 (Rossini et al., 2011, and current Fig. 1a). On one hand, we did not establish a minimum ear biomass for silking to take place (Borrás et al., 2007), but probably reduced biomass allocation in ears of *dominated* plants (Pagano and Maddonni, 2007; Rossini et al., 2011) did not allow their completely developed florets to reach silk exposure from the husks. Most non-silked plants corresponded to hybrid AX877, a trend that revealed its high sensitivity to abiotic stress (Echarte et al., 2004). On the other hand, negative effects of reduced plant growth on ear development were observed predominantly before silking – 200 °C d (Fig. 1b), i.e. before the start of active ear growth (Otegui and Bonhomme, 1998) and the critical period for kernel set (Fischer and Palmer, 1984). Therefore, delayed silking (Fig. 1b) and enhanced ASI of individual plants (Fig. 1c) seemed to be a

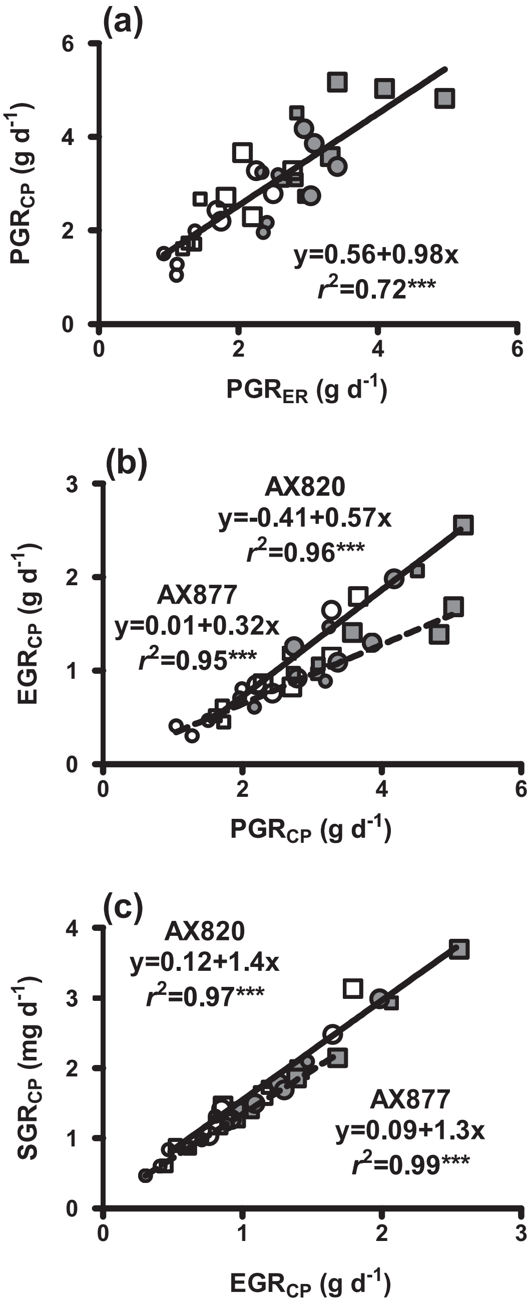

Fig. 2. Response of plant growth rate during the critical period (PGR$_{CP}$) to plant growth rate during early reproductive period (PGR$_{ER}$) (a), ear growth rate during the critical period (EGR$_{CP}$) to PGR$_{CP}$ (b), and spikelet growth rate during the critical period (SGR$_{CP}$) to EGR$_{CP}$ (c). Symbols as in Fig. 1. When significant differences were detected between hybrids ($P \leq 0.05$), solid and dotted lines represent fitted models for AX820 and AX877, respectively.

consequence of the effects of early plant growth on ear reproductive development (Fig. 1a), a link between growth and development that had not been reported previously.

The final number of completely developed florets per ear was reduced by N deficiency, a response that is supported by one previous research (Jacobs and Pearson, 1991) but not by others (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995b; Monneveux et al., 2005). This lack of consensus may be probably linked to more than one cause; for instance, genotypic variability in maximum number of completely developed florets per ear (largest for the modern temperate hybrids used in current research), or the approach used for computation of the number of completely developed florets per

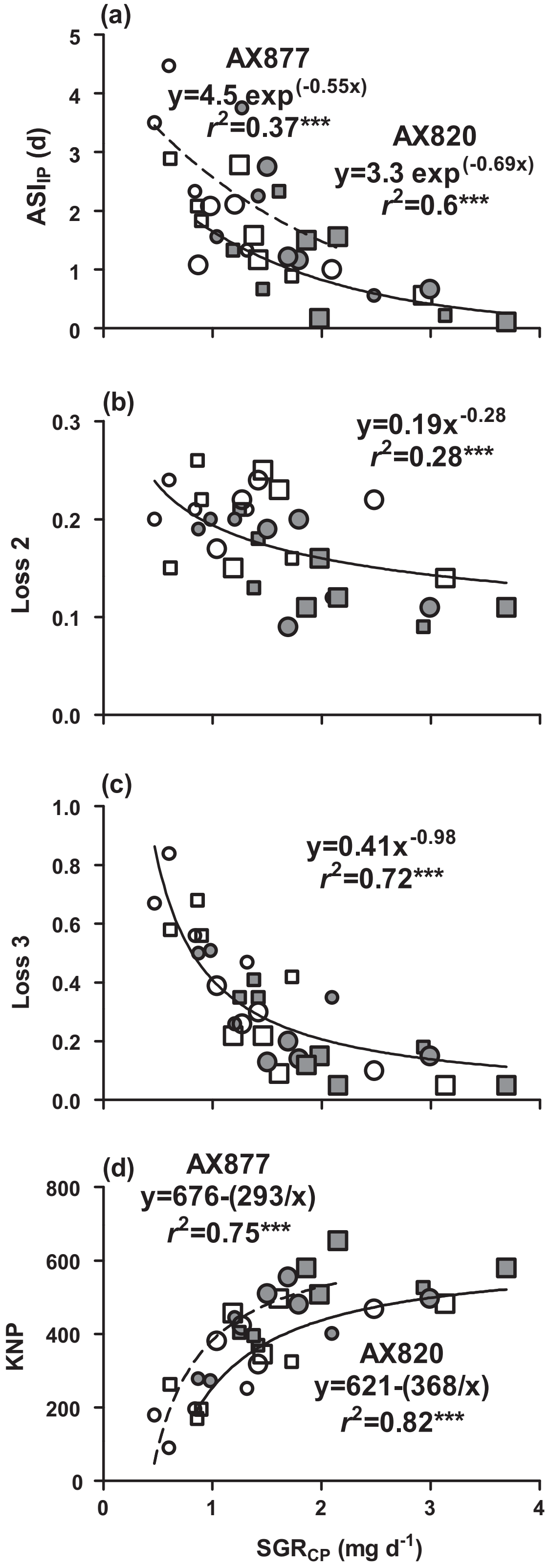

Fig. 3. The anthesis-silking interval at individual plant level (ASI$_{IP}$) (a), Loss 2 (b), Loss 3 (c) and kernel number per plant (KNP) as a function of spikelet growth rate during the critical period (SGR$_{CP}$). Symbols as in Fig. 1. When significant differences were detected between hybrids ($P \leq 0.05$), solid and dotted lines represent fitted models for AX820 and AX877, respectively.

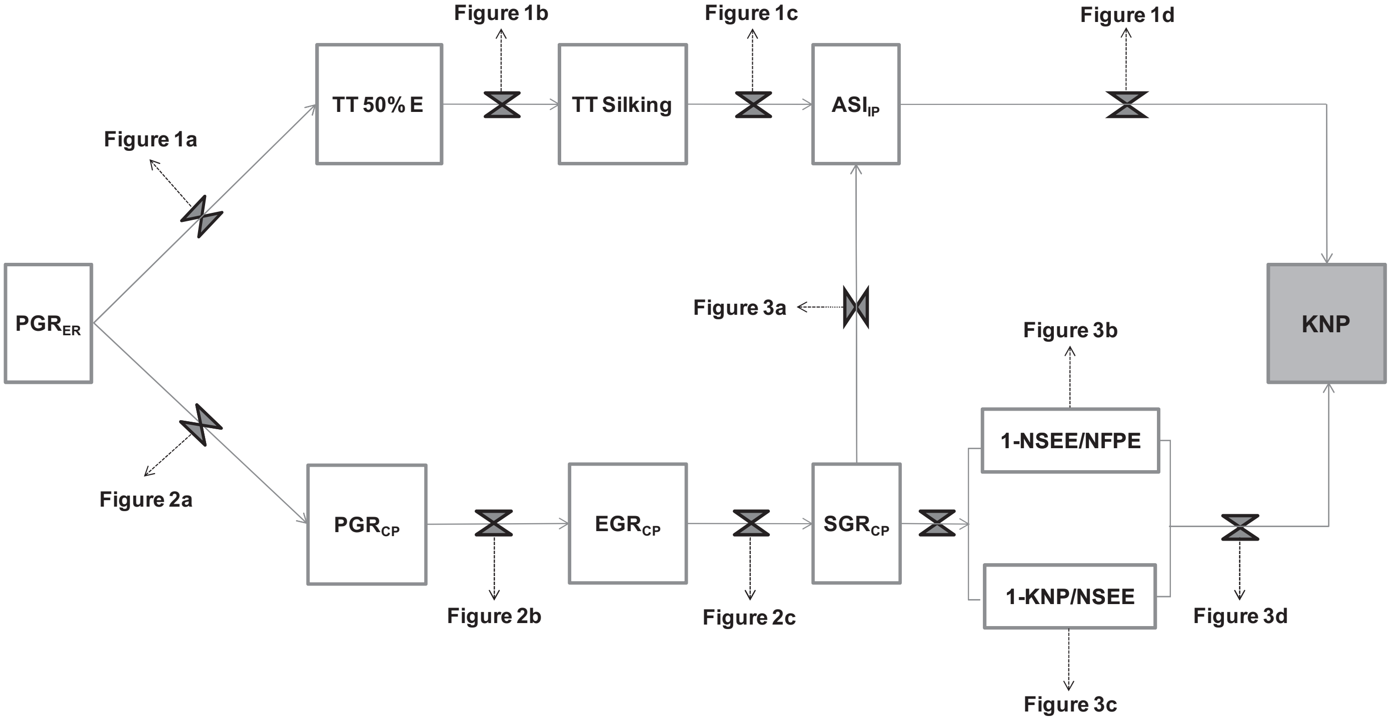

Fig. 4. Conceptual diagram describing the links between reproductive (upper part of the flow chart) and growth (lower part of the flow chart) traits leading to the determination of final kernel number per plant (KNP) in maize.

ear (average plant in all other reports vs. individual plant analysis in current research). Independently of these considerations, it is important to highlight that our findings on this trait did not rely exclusively on a single data set obtained from sampling at or near silking. They were supported by the analysis of floret development from early reproductive initiation at the uppermost axillary bud. Reduced N level caused a larger decline in ear development rate of progress among *dominated* than among *dominant* individuals (i.e. it desynchronized these two groups), a trend reported only in response to reduced irradiance per plant due to enhanced crowding (Otegui, 1997; Pagano et al., 2007). In previous research, however, ears at high stand density finally achieved a similar final number of completely developed florets per ear than that observed at normal plant populations, a condition never met between plants grown at N_0 and at N_{200} in the current research. Pagano et al. (2007) proposed an indirect effect of reduced plant growth (i.e. low assimilates availability) at high stand density on early ear development, because these effects disappeared in subsequent stages. Apparently, severe N stress experienced by plants grown at N_0 in current research caused a permanent negative effect of reduced growth on ear development (i.e. direct effect; Fig. 1a), which deserved further investigation. Nevertheless, this effect had minimum consequences on final reproductive performance, represented in the weak relationship between kernel number per plant and Loss 1.

The ASI of individual plants was a good indicator of the final reproductive performance (i.e. kernel number per plant) of different plant types in response to variable resource availability per plant produced by contrasting N × stand density combinations (Fig. 1d). The response pattern, however, was represented by a simple linear regression, and not by the negative exponential one suggested for variable water (Bolaños and Edmeades, 1993) or N regimes (D'Andrea et al., 2006). The cause of this apparent bias does not seem related to the use of ASI of individual plants (current research) instead of ASI of plant population (other sources), but to the range of ASI explored in each study. Our work did not include ASI values larger than 4.5 d, which were present in mentioned reports and forced the exponential fits. Moreover, the relationship between kernel number per plant and ASI (i) almost disappeared when only one data pair with ASI >10 d was removed from D'Andrea et al. (2006) analysis, or (ii) was better explained by a linear ($r^2 \cong 0.69$) rather than by an exponential function ($r^2 \cong 0.61$) for data with ASI < 10 d from Bolaños and Edmeades (1993) analysis. Nevertheless, our research did detect differences between tested hybrids, because both explored a similar range of kernel number per plant but very different ranges of ASI (toward shorter values of the interval for AX820 than for AX877). This trend is supported by previous findings based on maize populations with contrasting degree of breeding for tolerance to water deficit stress (Chapman and Edmeades, 1999), for which authors suggested that the observed reduction in ASI by plant population may have resulted from an enhanced flux of assimilates to the ear. Edmeades et al. (1993) reinforced this idea with the strong relationship established between the ASI of plant population and spikelet biomass at 50% anthesis. Our research strengthened it further with the relationship between ASI of individual plants and spikelet growth rate during the critical period (Fig. 3a), because the latter is a better proxy of assimilate flux to the ear than spikelet biomass at anthesis. Fitted models differed between hybrids, because plants of AX820 exhibited a larger range of spikelet growth rate during the critical period and a smaller range of ASI than AX877.

N stress had a weak effect on floret development, and consequently on the response of kernel number per plant to reductions in the number of completely developed florets per ear (i.e. to Loss 1). As opposed, delayed ear morphogenesis registered among *dominated* individuals had a clear negative effect on the proportion of silks exposed from completely developed florets (i.e. increased Loss 2), as previously observed for enhanced stand density (Pagano et al., 2007). This trend was linked to variations in spikelet growth rate during the critical period (Fig. 3b). Interestingly, hybrids in current research did not differ in the response of Loss 2 to spikelet growth rate during the critical period, and of kernel number per plant to Loss 2. For the latter, *dominated* plants exhibited a larger

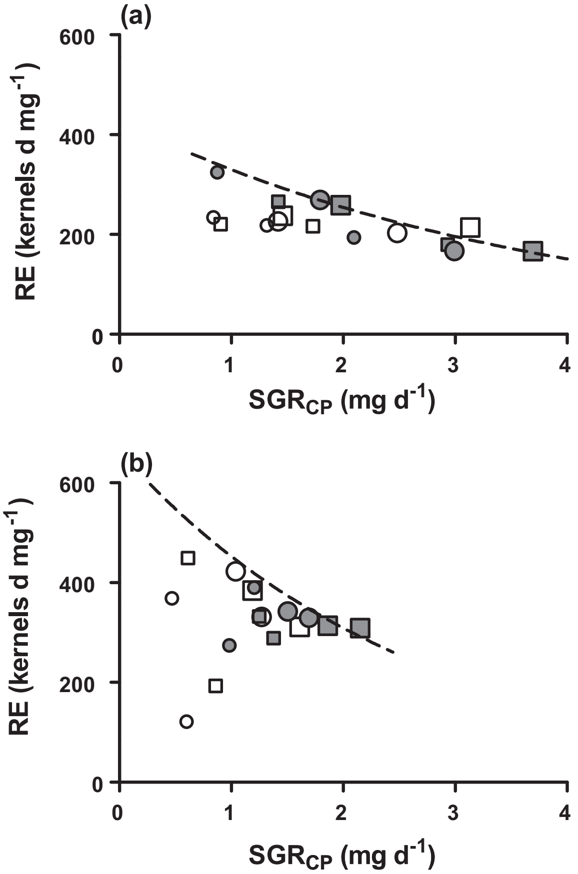

Fig. 5. Reproductive efficiency (RE) as a function of spikelet growth rate during the critical period (SGR_{CP}) of hybrids AX820 (a) and AX877 (b). Symbols as in Fig. 1. Dotted lines represent the boundary function for the relationship between RE and SGR_{CP} for AX820 ($y = 421\exp^{(-0.253x)}$; $r^2 = 0.99$; $P < 0.001$) and for AX877 ($y = 655\exp^{(-0.372x)}$; $r^2 = 0.97$; $P < 0.001$).

decrease in kernel number per plant than the *dominant* plants for a given increase in the proportion on non-exposed silks. This differential response may be indicative of an indirect effect of silking dynamics among florets within an ear, not related to pollination failure but to pollination asynchrony among florets (Cárcova et al., 2000; Uribelarrea et al., 2002; Lizaso et al., 2003). This asynchrony was larger for *dominated* than for *dominant* plants, and may have produced important reductions in final kernel numbers depending upon the time elapsed between fertilization of early- and late-exposed silks. Cárcova and Otegui (2001) demonstrated that a time lag of 2–4 d between pollination of successive cohorts of silks increased the proportion of fertilized ovaries that failed to set a kernel, i.e. increased kernel abortion (Loss 3).

Kernel abortion is usually indicated as the main determinant of observed variations in kernel number per plant of maize crops grown under abiotic stress conditions, but only a few studies made a precise quantification of this source of loss (Otegui et al., 1995; Rattalino Edreira et al., 2011). In the current research, contrasting plant categories were responsible of the large variation observed in this trait, which explained differences in final kernel number per plant very accurately. Hybrids did not differ in the magnitude of the response (i.e. had almost identical slopes for this relationship). The range registered for Loss 3, however, differed between hybrids (*AX877* > *AX820*), due to their different spikelet growth rates. Consequently, kernel number per plant of both hybrids had a positive curvilinear response to spikelet growth rate during the critical period (Fig. 3d). This response was almost identical to the usually reported curvilinear (Echarte and Tollenaar, 2006; Cicchino et al., 2010) or bilinear (Pagano and Maddonni, 2007; D'Andrea et al., 2008) response of kernel number per plant to ear growth rate during critical period, due to the tight relationship between spikelet growth rate and ear growth rate during that time (Fig. 2c). Therefore, the reproductive efficiency derived from the quotient between kernel number per plant and spikelet growth rate during the critical period, was also almost identical to that computed for the relationship between kernel number per plant and ear growth rate during the same phase (Vega et al., 2001; Rossini et al., 2011).

The relationship between reproductive efficiency and spikelet growth rate during the critical period (Fig. 5) gave important clues for understanding the effect of treatments on final kernel set of maize crops. First, because we could establish boundary functions representative of maximum reproductive efficiency of each hybrid, which were defined predominantly by the *dominant* individuals. Second, because *dominated* plants of AX877 exhibited a higher reduction in reproductive efficiency than those of AX820. Third, because *dominated* plants of both hybrids showed the largest reduction in reproductive efficiency under N stress. Genotypic differences in reproductive efficiency could be related to a different biomass allocation between vegetative (cob and husks) and reproductive tissues of the ear (i.e. spikelets), not identified with our estimator of assimilates supply to florets (i.e. the quotient between ear shoot growth rate and the number of completely developed florets per ear) around the critical period. The lower reproductive efficiency of *dominated* plants of AX877 suggested the occurrence of a disruption in the symmetry of assimilates allocation among florets within the ear (Ganeshaiah and Uma Shaanker, 1992). Delayed floret development and enhanced pollination asynchrony may contribute to the onset of a primigenic dominance (Bangerth, 1989) exerted by early pollinated ovaries on the late pollinated ones (Cárcova and Otegui, 2007), which causes an increase in kernel abortion (Cárcova and Otegui, 2001) and a reduction in reproductive efficiency (Vega et al., 2001). Additionally, the negative effect of N stress on the reproductive efficiency of *dominated* plants may be linked to the role of this nutrient on the ability to use carbon from the grains through the activity of enzymes involved in C and N metabolisms (Below et al., 2000). Hence, the hypothesis of this work was partially accepted, because at N_0 both hybrids exhibited a reduction in reproductive efficiency not directly linked to reduced spikelet growth rate.

5. Conclusions

In the current research we established a link between plant growth before the critical period and final kernel number per plant, and described the relationships between different developmental and growth variables along the successive paths. The negative effect of N deficiency on plant growth was evident as a delay in the rate of floret development in the ear, which caused a delay in silking of each plant. This trend had a negative effect on flowering dynamics (enhanced ASI of the plants) and the silking pattern of individual ears, formerly related to kernel set. In spite of the weak effects of N on final floret development along the ear and the lack of response of kernel number per plant to this trait, delayed ear morphogenesis had a clear effect on the silking pattern. Hybrids, however, did not differ in the response to spikelet growth rate during the critical period of (i) the proportion of completely developed florets per ear that did not expose silks, and (ii) the abortion of fertilized florets. The reduced reproductive efficiency reported for hybrid AX877 under N stress in a previous research was primarily due to the abortion of fertilized ovaries, predominantly among *dominated* plants. A similar pattern was observed for the *dominated* plants of the other analyzed hybrid. These responses seemed not mediated by reduced assimilates flux to ear. They may be modulated directly by N availability and its effects on the capacity of a fertilized ovary for setting a kernel, i.e. on sink strength.

Acknowledgements

Authors wish to thank M. Chintio, L. Mayer, L. Blanco and A. Cirilo for their valuable help. This work was supported by the University of Buenos Aires (UBACyTG070), the National Agency for the Promotion of Science and Technology (ANPCyT PICT483) and the National Council of Research (CONICET) of Argentina (PIP 00125). M.A. Rossini holds a grant from CONICET, and G.A. Maddonni and M.E. Otegui are members of CONICET.

References

Andrade, F.H., Echarte, L., Rizzalli, R., Della Maggiora, A., Casanovas, M., 2002. Kernel number prediction in maize under nitrogen or water stress. Crop Sci. 42, 1173–1179.

Andrade, F.H., Vega, C.R.C., Uhart, S., Cirilo, A., Cantarero, M., Valentinuz, O., 1999. Kernel number determination in maize. Crop Sci. 39, 453–459.

Bangerth, F., 1989. Dominance among fruits/sinks and the search for a correlative signal. Physiol. Plant 76, 608–614.

Bassetti, P., Westgate, M.E., 1993. Water deficit affects receptivity of maize silks. Crop Sci. 33, 279–282.

Bänziger, M., Laffite, H.R., 1997. Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Sci. 37, 1110–1117.

Below, F.E., Cazzetta, J.O., Seebauer, J.R., 2000. Carbon/nitrogen interactions during ear and kernel development of maize. In: Westgate, M., Boote, K. (Eds.), Physiology and Modeling Kernel Set in Maize. CSSA Spec. Publ. 29. CSSA, Madison, WI, pp. 15–24.

Bolaños, J., Edmeades, G.O., 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. II. Responses in reproductive behavior. Field Crops Res. 31, 253–268.

Boomsma, C.R., Santini, J.B., Tollenaar, M., Vyn, T.J., 2009. Maize morphophysiological responses to intense crowding and low nitrogen availability: an analysis and review. Agron. J. 101, 1426–1452.

Borrás, L., Astini, J.P., Westgate, M.E., Severini, A.D., 2009. Modeling anthesis to silking in maize using a plant biomass framework. Crop Sci. 49, 937–948.

Borrás, L., Westgate, M.E., Astini, J.P., Echarte, L., 2007. Coupling time to silking with plant growth rate in maize. Field Crops Res. 102, 73–85.

Boyle, M.G., Boyer, J.S., Morgan, P.W., 1991. Stem infusion of liquid culture medium prevents reproductive failure of maize at low water potential. Crop Sci. 31, 1246–1252.

Cárcova, J., Otegui, M.E., 2001. Ear temperature and pollination timing effects on maize kernel set. Crop Sci. 41, 1809–1815.

Cárcova, J., Otegui, M.E., 2007. Ovary growth and maize kernel set. Crop Sci. 47, 1104–1110.

Cárcova, J., Uribelarrea, M., Borrás, L., Otegui, M.E., Westgate, M.E., 2000. Synchronous pollination within and between ears improves kernel set in maize. Crop Sci. 40, 1056–1061.

Caviglia, O.P., Melchiori, R.J.M., 2011. Contribution of contrasting plant hierarchies to the response to N fertilizer in maize. Field Crops Res. 122, 131–139.

Chapman, S., Edmeades, G.O., 1999. Selection improves drought tolerance in tropical maize populations. II. Direct and correlated responses among secondary traits. Crop Sci. 39, 1315–1324.

Cicchino, M., Rattalino Edreira, J.I., Uribelarrea, M., Otegui, M.E., 2010. Heat stress in field grown maize: response of physiological determinants of grain yield. Crop Sci. 50, 1438–1448.

Cirilo, A.G., Andrade, F.H., 1994. Sowing date and maize productivity. I. Crop growth and dry matter partitioning. Crop Sci. 34, 1039–1043.

D'Andrea, K.E., Otegui, M.E., Cirilo, G.A., 2008. Kernel number determination differs among maize hybrids in response to nitrogen. Field Crops Res. 105, 228–239.

D'Andrea, K.E., Otegui, M.E., Cirilo, A.G., Eyhérabide, G., 2006. Genotypic variability in morphological and physiological traits among maize inbred lines. Nitrogen responses. Crop Sci. 46, 1266–1276.

D'Andrea, K.E., Otegui, M.E., Cirilo, A.G., Eyhérabide, G., 2009. Ecophysiological traits in maize hybrids and their parental inbred lines: phenotyping of responses to contrasting nitrogen supply levels. Field Crops Res. 114, 147–158.

Echarte, L., Andrade, F.H., Vega, C.R.C., Tollenaar, M., 2004. Kernel number determination in Argentinean maize hybrids released between 1965 and 1993. Crop Sci. 44, 1654–1661.

Echarte, L., Tollenaar, M., 2006. Kernel set in maize hybrids and inbred lines exposed to stress. Crop Sci. 46, 870–878.

Edmeades, G.O., Bolaños, J., Hernández, M., Bello, S., 1993. Causes for silk delay in a lowland tropical maize population. Crop Sci. 33, 1029–1035.

Edmeades, G.O., Daynard, T.B., 1979. The development of plant-to-plant variability in maize at different planting densities. Can. J. Plant Sci. 59, 561–576.

Fischer, K.S., Palmer, F.E., 1984. Tropical maize. In: Goldsworthy, P.R., Fischer, N.M. (Eds.), The Physiology of Tropical Field Crops. John Wiley & Sons, Chichester, England, pp. 213–248.

Ganeshaiah, K.N., Uma Shaanker, R., 1992. Frequency distribution of seed number per fruit in plants: a consequence of the self-organizing process? Curr. Sci. 62, 359–365.

Hall, A.J., Ginzo, H.D., Lemcoff, J.H., Soriano, A., 1980. Influence of drought during pollen shedding on flowering growth and yield of maize. Agron. J. Crop Sci. 149, 287–298.

Hall, A.J., Lemcoff, J.H., Trápani, N., 1981. Water stress before and during flowering in maize and its effects on yields, its components, and their determinants. Maydica 26, 19–38.

Hall, A.J., Vilella, F., Trapani, N., Chimenti, C., 1982. The effects of water stress and genotype on the dynamics of pollen shedding in maize. Field Crops Res. 5, 349–363.

Jacobs, B.C., Pearson, C.J., 1991. Potential yield of maize determined by rates of growth and development of ears. Field Crops Res. 27, 281–298.

Jandel TBLCURVE, 1992. TableCurve 3.0. Curve Fitting Software. Jandel Scientific, Corte Madera, CA.

Lemcoff, J.H., Loomis, R.S., 1986. Nitrogen influences on yield determination in maize. Crop Sci. 26, 1017–1022.

Lizaso, J.I., Ritchie, J.T., 1997. Maize shoot and root response to root zone saturation during vegetative growth. Agron. J. 89, 125–134.

Lizaso, J.I., Westgate, M.E., Batchelor, W.D., Fonseca, A., 2003. Predicting potential kernel set in maize from simple flowering characteristics. Crop Sci. 43, 892–903.

Maddonni, G.A., Cárcova, J., Iglesias Pérez, M.E., Ghersa, C.M., 1999. Maize flowering dynamic in soils with contrasting agricultural history. Maydica 44, 0–7.

Maddonni, G.A., Otegui, M.E., 2004. Intra-specific competition in maize: early-determined hierarchies among plants affect final kernel set. Field Crops Res. 85, 1–13.

Monneveux, P., Zaidi, P.H., Sanchez, C., 2005. Population density and low nitrogen affects yield-associated traits in tropical maize. Crop Sci. 45, 535–545.

Otegui, M.E., 1997. Kernel set and flower synchrony within the ear of maize. II. Plant population effects. Crop Sci. 37, 448–455.

Otegui, M.E., Andrade, F.H., Suero, E.E., 1995. Growth, water use, and kernel abortion of maize subjected to drought at silking. Field Crops Res. 40, 87–94.

Otegui, M.E., Bonhomme, R., 1998. Grain yield components in maize. I. Ear growth and kernel set. Field Crops Res. 56, 247–256.

Otegui, M.E., Melón, S., 1997. Kernel set and flower synchrony within the ear of maize. I. Sowing date effects. Crop Sci. 37, 441–447.

Pagano, E., Cela, S., Maddonni, G.A., Otegui, M.E., 2007. Intra-specific competition in maize: ear development flowering dynamics and kernel set of early-established plant hierarchies. Field Crops Res. 102, 198–209.

Pagano, E., Maddonni, G.A., 2007. Intra-specific competition in maize: early established hierarchies differ in plant growth and biomass partitioning to the ear around silking. Field Crops Res. 101, 306–320.

Pommel, B., 1990. Influence du poids de la semence et de la profondeur de semis sur la croissance et le développement de la plantule de maïs. Agronomie 10, 699–708.

Ritchie, S.W., Hanway, J.J., 1982. How a Corn Plant Develops. Iowa State Univ. Special Report 48.

Ritchie, J.T., NeSmith, D.S., 1991. Temperature and crop development. In: Hanks, J., Ritchie, J.T. (Eds.), Modelling Plant and Soil Systems. Agronomy Series, vol. 31. American Society of Agriculture, Crop Science Society of America, Soil Science Society of America, Madison, WI, pp. 5–29.

Rattalino Edreira, J.I., Budakli Carpici, E., Sammarro, D., Otegui, M.E., 2011. Kernel set of temperate and tropical maize hybrids exposed to heat stress around flowering. Field Crops Res. 123, 62–73.

Rossini, M.A., Maddonni, G.A., Otegui, M.E., 2011. Inter-plant competition for resources in maize crops grown under contrasting nitrogen supply and density: variability in plant and ear growth. Field Crops Res. 121, 373–380.

Sangoi, L., Gracietti, M.A., Rampazzo, C., Bianchetti, P., 2002. Response of Brazilian maize hybrids from different eras to changes in plant density. Field Crops Res. 79, 39–51.

Schussler, J.R., Westgate, M.E., 1991. Maize kernel set at low water potential. I. Sensitivity to reduced assimilates during early kernel growth. Crop Sci. 31, 1189–1195.

Schussler, J.R., Westgate, M.E., 1994. Assimilate flux determines kernel set at low water potential in maize. Crop Sci. 35, 1074–1080.

Uhart, S.A., Andrade, F.H., 1995a. Nitrogen deficiency in maize. I. Effects on crop growth development, dry matter partitioning, and kernel set. Crop Sci. 35, 1376–1383.

Uhart, S.A., Andrade, F.H., 1995b. Nitrogen deficiency in maize II. Carbon–Nitrogen interaction effects on kernel number and grain yield. Crop Sci. 35, 1384–1389.

Uribelarrea, M., Cárcova, J., Otegui, M.E., Westgate, M.E., 2002. Pollen production pollination dynamics, and kernel set in maize. Crop Sci. 42, 1910–1919.

Vega, C.R.C., Andrade, F.H., Sadras, V.O., 2001. Reproductive partitioning and seed set efficiency in soybean, sunflower and maize. Field Crops Res. 72, 163–175.

Westgate, M.E., Boyer, J.S., 1986. Reproduction at low silk and pollen water potentials in maize. Crop Sci. 26, 951–956.

Zinselmeier, C., Lauer, M.J., Boyer, J.S., 1995. Reversing drought-induced losses in grain yield: sucrose maintains embryo growth. Crop Sci. 35, 1390–1400.