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RESEARCH PAPER

Artificial selection for grain yield has increased net CO_2 exchange of the ear leaf in maize crops

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Abstract

Identifying the physiological traits indirectly selected during the search for high-yielding maize hybrids is useful for guiding further improvements. To investigate such traits, in this study we focused on the critical period of kernel formation because kernel number is the main yield component affected by breeding. Our results show that breeding has increased the number of florets per ear and ear growth rate but not the vegetative shoot growth rate, suggesting localised effects around the ear. Consistent with this possibility, breeding has increased the net CO_2 exchange of the ear leaf in field-grown crops grown at high population densities. This response is largely accounted for by increased light interception (which increases photosynthesis) and by reduced rates of respiration of the ear leaf in modern hybrids compared to older ones. Modern hybrids show increased ear-leaf area per unit leaf dry matter (specific leaf area), which accounts for the reduced respiratory load per unit leaf area. These observations are consistent with a model where the improved ear leaf CO_2 exchange helps the additional florets produced by modern hybrids to survive the critical period of high susceptibility to stress and hence to produce kernels.

Keywords: Breeding, canopy density, carbon balance, maize, photosynthesis, respiration, specific leaf area, *Zea mays*.

Abbreviations: PAR, photosynthetically active radiation; PP, plant population; SLA, specific leaf area; YOR, year of release.

Introduction

Maize (*Zea mays*) represents 45% of global grain production, with a contribution of 1147 million tons per year in 2018 (http://www.fao.org/faostat). Maize grain yields per unit area have increased gradually over time, thanks to improvements in both management practices and genetics (Duvick, 2005*a*). The most relevant practices include the use of new, improved technologies for the control of weeds, insects, and diseases, together with improved harvest management, the application of fertilisers (including synthetic nitrogen), better timeliness of sowing dates, and increases in crop plant population (PP) densities at sowing (Tollenaar and Lee, 2002). The genetic improvements can be summarised as increased stability of grain yield under a wide range of stressful conditions such as drought events, temperature extremes, and high PPs (Russell, 1986; Tollenaar and Wu, 1999; Tollenaar and Lee, 2002; Duvick, 2005*b*; Hernández *et al.*, 2014).

Maize grain yield has a parabolic response to increasing PP, with yield decreases both below and above the optimum value (Duncan, 1958; Sarlangue *et al.*, 2007). When crops are grown at supra-optimal PP, the additional plants do not compensate for the reductions in kernel number per plant and therefore crop grain yield falls (Deng *et al.*, 2012). Plotting grain yield against the year of release (YOR) of cultivated hybrids to the market provides a description of the impact of breeding. Modern maize hybrids have increased tolerance to high PP, as revealed by a steeper response of grain yield to YOR in a high PP (7.9 plants m^{-2}) than in a low PP (1 plant m^{-2}) (Duvick, 2005*a*). As a result of this, old hybrids from North and South America reach their maximum grain yield at lower PP than modern hybrids (Russell, 1986; Tollenaar and Lee, 2002; Echarte *et al.*, 2004; Luque *et al.*, 2006; Duvick *et al.*, 2010; Di Matteo *et al.*, 2016; Gonzalez *et al.*, 2018).

The enhanced grain yield of modern hybrids at high PP is largely accounted for by changes in the number of kernels at harvest (Echarte *et al.*, 2000; Tollenaar and Lee, 2002; Luque *et al.*, 2006; Ciancio *et al.*, 2016; Borrás and Vitantonio-Mazzini, 2018). The critical period for determination of kernel number is a ~30-d interval centred at silking (Fischer and Palmer, 1984; Cerrudo *et al.*, 2013), when the reproductive organs of the plant are more sensitive to stressful events (Tollenaar and Daynard, 1978; Kiniry and Ritchie, 1985). Breeding has consistently increased ear growth capacity during this critical period (Echarte *et al.*, 2004; Luque *et al.*, 2006; Ciancio *et al.*, 2016); however, whether more kernels at harvest results from the formation of more florets, improved silking, or reduced abortion remains unknown.

Modern hybrids may show higher (Dwyer *et al.*, 1991), similar (Duvick, 1997), or slightly lower (Perez *et al.*, 2019) plant leaf area than older genotypes and there are no major trends in whole-canopy light interception (Tollenaar and Aguilera, 1992; Lee and Tollenaar, 2007; Echarte *et al.*, 2013; Zhao *et al.*, 2015; Perez *et al.*, 2019; Curin *et al.*, 2020). However, modern hybrids tend to have more erect leaves (Crosbie and Mock, 1981; Meghji *et al.*, 1984; Russell, 1991; Boomsma *et al.*, 2009; Duvick *et al.*, 2010; Ma *et al.*, 2014; Perez *et al.*, 2019) and higher leaf area located at lower relative positions in the canopy (Perez *et al.*, 2019). These architectural traits reduce the photosynthetically active radiation (PAR) absorbed by upper strata of the canopy and increase the PAR reaching lower strata (Ma *et al.*, 2014; Zhao *et al.*, 2015; Perez *et al.*, 2019), which might be beneficial for whole-canopy photosynthesis (Long *et al.*, 2006). However, whether breeding has affected vegetative shoot growth capacity during the critical period is not clear.

Studies on the effects of breeding for higher yields in maize have focused more on plant architecture and morphological traits than on physiological processes (Sarquís *et al.*, 1998). For instance, our knowledge about the impact of breeding on carbon balance is limited in at least in two respects. First, information for carbon balance during the critical period is scant. There are measurements of leaf net CO_2 exchange at elevated levels of PAR (2000 μmol m^{-2} s^{-1}) during the post-silking period, when modern hybrids have higher rates than older ones (Dwyer and Tollenaar, 1989; Dwyer *et al.*, 1991; Earl and Tollenaar, 1999; Richards, 2000; Lee and Tollenaar, 2007; Li *et al.*, 2019). Enhanced photosynthesis post-silking contributes to the filling of a larger number of kernels without a reduction in dry weight per kernel (Tollenaar and Aguilera, 1992; Luque *et al.*, 2006; Zhao *et al.*, 2015), but not to the breeding effects on kernel number. Luque *et al.*, (2006) observed a positive relationship between radiation use efficiency during the critical period and YOR, which suggests that changes in carbon balance may have taken place. Second, whilst increasing PP exacerbates mutual shading among plants, the impact of breeding and selection for yield on net CO_2 exchange at low PAR has received little attention. Nissanka *et al.* (1997) observed lower rates of respiration per unit CO_2 fixed in a new hybrid compared to an old one grown under controlled conditions. However, in contrast when Earl and Tollenaar (1998) measured respiration in disks taken from the leaves of six maize hybrids and incubated in a water bath for up to 120 min, they found no clear correlation with YOR. This lack of agreement, together with the proposal that reducing the respiratory load will provide a feasible strategy to boost the yield of crops (Amthor *et al.*, 2019), highlights the importance of reassessing this trait using more modern techniques.

The improved performance of modern hybrids at high PPs (Echarte *et al.*, 2004; Duvick, 2005*b*; Luque *et al.*, 2006) is a key component of the steady increase in global grain yield of maize that has occurred since the widespread adoption of hybrids in the 1960s (Ray *et al.*, 2013). Knowing the mechanisms involved in the higher tolerance of modern hybrids to the deeper shading of high PP canopies would help to guide further yield improvements in maize and other crops. The aim of this study was therefore to address the following questions: First, are the changes in kernel number caused by breeding the result of more florets and/or of reduced kernel abortion?

Second, are changes in ear growth accompanied by changes in vegetative shoot growth (which would indicate differential overall growth capacity)? Third, has net CO_2 exchange of the ear leaf during the critical period in high PP crops been affected by breeding?

Materials and methods

Plant material

The maize (*Zea mays*) hybrids used in this study together with their year of release (YOR) to the Argentinian market were as follows: DKF880 (1965), M400 (1978), DK4F32 (1980), DK3F22 (1983), Maizal 86 (1985), DK4F37 (1988), DK664 (1993), DK752 (1993), C280 (1995), DK757 (1997), DK696 (1997), DK688 (1997), DK682 (2000), AW190 (2003), DK747 (2004), DK690 (2004), DK699 (2007), DK692 (2009), and DK7210 (2012). The hybrids that were used in each individual experiment are listed in Supplementary Table S1. The criteria for the inclusion of these genotypes were that they are all temperate hybrids and that they have all been successfully established in the Argentinean market. The hybrids mostly originate from the same breeding company (Dekalb/Cargill, except the genotype M400 that belongs to Morgan). One experiment (Exp. 5) was conducted with multiple aims and included hybrids that did not fulfil the criterion of wide acceptance, namely DK2F10 (1980), DK7020 (2015), DK7320 (2015), and DK7270 (2018). Although these hybrids were not used for the analysis of regression against YOR, their results were informative and hence are also reported.

Growth conditions and experimental design

In Exp. 1, commercial maize hybrids were sown in a deep silty clay loam soil (Vertic Argiudol) at the experimental field of the Faculty of Agronomy of the University of Buenos Aires (34°35′S; 58°29′W), in October 2016. We used a split-plot experimental design. We assigned plant population (PP; two levels, three replicates) to the main plots and the hybrids to the sub-plots. Each sub-plot was 10 m long with four rows separated by 0.5 m, and only the central rows were used for measurements. The high PP treatment was 8 plants m^{-2} (typical of commercial crops in Argentina in high-yield environments) and the low PP treatment was 4 plants m^{-2}.

In Exps 2–4, the plants were cultivated in 4-l pots containing peat moss and sand (1:1) either in a glasshouse (Exps 2 and 3) or in the field (Exp. 4), at the Faculty of Agronomy of the University of Buenos Aires. We used three seeds per pot and thinned the seedlings to one per pot. We used three pots (sub-replicates) per hybrid per experiment (replicate), which were arranged forming a line to reach a PP of 6 plants m^{-2}. Experiments 2, 3, and 4 were conducted in August, October, and November 2018, respectively. Respiration measurements were done at the V5 stage (Ritchie *et al.*, 1993).

In Exp. 5, hybrids were sown in October 2019 in the experimental field of the INTA station, located at Pergamino (33°56′S; 60°33′W). The soil was a silty clay loam (Typic Argiudol) of more than 2 m depth and 3.0% topsoil organic matter. We used three blocks (replicates) and each plot within a given block was 6 m long with five rows separated by 0.52 m. The PP was 9 plants m^{-2}.

In Exps 6 and 7, hybrids were sown in October 1996 and 1997, respectively, at the INTA Pergamino station. Some data from these experiments were published by Luque *et al.* (2006); here, we present previously unpublished data corresponding to a PP of 9 plants m^{-2} (high, but not supra-optimal) and additional PPs are included only in Supplementary Fig. S1. The experimental design was split-plot with three replicates, and each plot within a given block was 10 m long with five rows separated by 0.7 m.

To ensure that growth potential could be fulfilled, water was provided as needed, and the plants were kept free of diseases, weeds, and herbivorous insects. Field crops were fertilised with either 20 g N m^{-2} (Exps 1, 5), 22 g N m^{-2} (Exp. 6), or 23 g N m^{-2} (Exp. 7), and plants in pots were fertilised with adequate N via irrigation. Environmental conditions are detailed in Supplementary Table S2A.

Yield components

Adjacent plants were tagged (10 in Exps 1, 6, and 7; five in Exp. 5) in the central row of each plot (three replicate plots) and then harvested at physiological maturity to obtain final grain yield (expressed as the equivalent t ha^{-1}) and total kernel number (grains m^{-2}). The tagged plants were individually harvested and weighed after being dried under forced-air circulation at 65 °C (Parco *et al.*, 2020). In addition, in Exp. 1, four ears per plot (three replicate plots) were harvested 4 d after the first silk became visibly exposed in order to count the number of florets and silked florets. The number of exposed silks per ear defined the number of florets with emerged silks. The total number of florets was defined as the sum of florets with emerged silk plus the florets with non-exposed silks with a length of at least 3 mm. The abortion rate was calculated as the percentage of total fertilised ovules (estimated as the number of florets with exposed silk; Cárcova *et al.*, 2000) that set kernels at physiological maturity. In Exps 6 and 7, 10 ears per plot (three replicate plots) were harvested. Floret number was calculated as the product of the number of rows recorded at the centre of the ear and the number of spikelets per ear counted in two opposite rows per ear at silking (Luque *et al.*, 2006).

Light profile

We measured PAR using a line quantum-sensor (2013-dual, Cavadevices, Buenos Aires, Argentina) above the canopy (incident PAR) and within the canopy. Five measurements were conducted per plot (three replicate plots) at clear middays during the critical period of kernel formation in Exps 1 and 5. Relative PAR (%) was calculated as the percentage of PAR measured within the canopy relative to PAR measured above the canopy. In Exp. 1, the sensor was placed transversal to the rows in three strata of the canopy: bottom (lowermost green leaf), middle (ear leaf), and upper (third leaf from the tassel). In Exp. 5, the sensor was placed parallel to the row, ~10 cm away from the plant and at the height of the ear leaf.

Net CO_2 exchange

To measure net CO_2-exchange, stomatal conductance, and the leaf intercellular and atmospheric concentrations of CO_2, we used a LI-COR 6400 portable gas-exchange system. We also recorded leaf temperature, air temperature, and relative humidity during the measurements (Supplementary Table S2B). In Exp. 1, we obtained curves for net CO_2 exchange versus PAR (0, 50, 100, 300, 600, 900, 1500, and 2000 µmol m^{-2} s^{-1}) using a 6400-02B LED light-source chamber (Supplementary Table S3). Air flow and CO_2 concentration in the reference cell (CO_2R) were controlled automatically at 300 µmol s^{-1} and 400 ppm, respectively. Measurements were made on the ear leaf at clear middays at 2 d after silking (i.e. stage R1, within the critical period for determination of kernel number; Cerrudo *et al.*, 2013). We took measurements on one plant in each plot, with three replicate plots per genotype and per PP. In Exp. 5, we took measurements on the ear leaf of three plants in each plot, with three replicate plots per genotype close to midday by setting the PAR provided by the built-in source at the values registered simultaneously in the field. In Exps 2–4, we measured the rate of respiration at midday as net CO_2 exchange at a PAR of 0 µmol m^{-2} s^{-1} on the fourth leaf at the V5 stage (Ritchie *et al.*, 1993) in three replicate plants per hybrid per experiment. In this case we reduced the air flow to 200 µmol s^{-1} to increase the accuracy of the

measurements. In Exp. 1, each curve took ~20 min to record, and the final curve was obtained in three blocks to minimise deviations from midday (the first was taken immediately before midday, the second immediately after midday, and the third immediately before midday on the following day). In Exps 2–5, the point measurements took ~5 min. (Exp. 5 included one block immediately before midday and the other immediately after midday). The order of measurement of the hybrids was assigned at random within each block.

Growth measurements

In Exps 3, 1 and 5, we used a non-destructive allometric model (Supplementary Table S4; Vega *et al.*, 2001; Ciancio *et al.*, 2016) to calculate biomass during the critical period, and from that we calculated the vegetative shoot growth rate and the growth rate of the uppermost ear. Briefly, in the three replicate plots we tagged adjacent plants (sub-replicates) in the middle of the row of each plot (10 in Exp. 1, five in Exp. 5). We then measured the basal stem diameter (average between maximum and minimum), plant height (from ground level to the collar of the last expanded leaf), and maximum ear diameter. Measurements were performed at 15 d before silking of the apical ear (stem diameter and plant height only) and silking (i.e. at least one extruded silk visible), and at 15 d after silking (all traits). For measurements prior to silking, we predicted the date using a thermal-time method in combination with historical records of daily temperature means for the site of the experiment. To parameterise the model, we harvested an independent set of plants on the same dates and determined the biomasses of the ears and other above-ground organs after drying at 70 °C for 72 h. In Exps 6 and 7, we directly measured biomass by harvesting the shoots of six plants per plot (three replicate plots) at 7 d before silking and 20 d after silking and separating them into ears and vegetative organs. Samples were dried at 60 °C for 10 d before weighing. In Exps 6 and 7, when leaves had completed expansion leaf area was calculated as the product of leaf length and maximum leaf width and corrected by factor 0.75 (Montgomery, 1911).

Chlorophyll content

We used a SPAD-502 chlorophyll meter (Minolta) in combination with calibration curves fitted separately for each hybrid (Supplementary Table S5) to estimate chlorophyll contents (μg cm^{-2}). In Exp. 1, we obtained SPAD readings at stageR1 from three plants (sub-replicates) per plot (three replicate plots) at three different canopy strata: bottom (lowermost green leaf), middle (ear leaf), and upper (third leaf from the tassel). Measurements were performed at three different positions along the lamina: basal (close to main stem), middle, and apical (distal to main stem). To construct the calibration curves, we measured chlorophyll contents in 12 leaf disks (1 cm diameter) extracted in 2 ml N,N-dimethylformamide (72 h in darkness at 4 °C), and measured absorbance at 647 nm and 660 nm (Moran, 1982) using a spectroradiometer (Ultrospec 2100 pro, Amersham Biosciences).

Specific leaf area

In Exps 3, 1 and 5, we harvested 16 leaf disks from three plants (sub-replicates) per plot (three replicate plots) at 2 d after silking in three canopy strata: bottom (lowermost green leaf), middle (ear leaf), and upper (third leaf from the tassel). We calculated specific leaf area (SLA, $cm^2\ g^{-1}$) as the ratio between disk area and dry weight (weighed after 48 h at 70 °C). In Exp. 6, we calculated the SLA in the stratum containing the ear as the ratio between leaf area and leaf dry weight within that stratum. In this case, we calculated leaf area as described above and determined dry weigh after 10 d at 60 °C.

Statistical analysis

The basic data used for the analyses are shown in Supplementary Tables S6 (Exp. 1), S7 (Exps 2–4), S8 (Exp. 5), S9 (Exp. 6), and S10 (Exp.7). A summary of the response variables measured in each experiment is shown in Supplementary Table S11. A list of explanatory variables incorporated in each analysis is given in Supplementary Table S12. The genetic component, which was the main focus in most of the experiments, was incorporated into the variable YOR because we wanted to investigate the trends caused by breeding. We used linear regression with a step-wise model for multiple regression in the InfoStat software (http://www.infostat.com.ar) to investigate the significance of the relevant variables and their interactions, and to obtain R^2 values (Supplementary Table 12). In the analysis of yield components in relation to YOR ranging from 1965–2018, we tested each variable in multiple linear regressions to investigate whether the slope of the response was different for YORs earlier than 1990 compared to later (Supplementary Table 12). We set the threshold at 1990 because the introduction into the market of single-cross hybrids in Argentina took place around that time. When different experiments were pooled in a single analysis, the effect of each experiment was incorporated into the analysis of regression by means of categorical variables (dummy variables). In some cases we normalised the data to the mean of the hybrids grown at high or low PP, to the average of the mean, or to the mean of the experiment. This procedure was done to facilitate the visualisation of the trends of interest without the interference caused by PP (Exp. 1), replicate time (Exp. 5), or experiment date (Exps 2–4).

Results

Historical trend of plant populations used in maize breeding

The higher optimum PP for grain yield in modern compared to older hybrids (Russell, 1986; Tollenaar and Lee, 2002; Echarte *et al.*, 2004; Luque *et al.*, 2006), has presumably been the result of artificial selection for grain yield performed at high PPs by breeders (Eyherabide *et al.*, 1995; Troyer, 1996; Tokatlidis and Koutroubas, 2004), but a quantitative description of this trend is lacking. As a proxy to obtain quantitative information about the historical trend of the conditions used for hybrid selection in breeding programs in Argentina, we searched for local papers studying optimal grain yield and recorded the PP (Supplementary Table S13). The rationale behind the use of this proxy is that these reports were often presented by the breeders themselves or by researchers connected to the breeders. We found that the PP used for maximum yield tests had increased over the last six decades at a rate of ~0.86 plants m^{-2} $decade^{-1}$ (Fig. 1). Despite the fact that PP increased significantly between 1962–1990 and 1990–2018 (4.8±0.2 versus 8.0±0.2 plants m^{-2}, mean ±SE), grain yields per plant did not decrease (143±8 versus 163±9 g $plant^{-1}$; $P>0.05$, Supplementary Table S13). This lack of yield penalty (Sarlangue *et al.*, 2007; Deng *et al.*, 2012) reflects the higher tolerance to PP of modern genotypes (Russell, 1986; Tollenaar and Lee, 2002; Echarte *et al.*, 2004; Duvick, 2005*a*; Luque *et al.*, 2006; Duvick *et al.*, 2010; Di Matteo *et al.*, 2016; Gonzalez

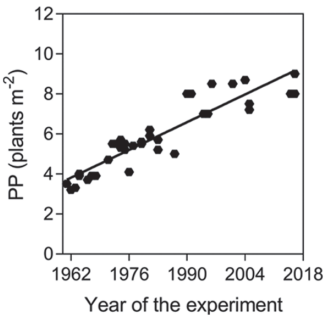

Fig. 1. Maize breeding has been conducted under increasing plant population (PP) densities over the recent decades. The PPs used in published experiments to test maize hybrids in Argentina are plotted against the year when each experiment was conducted. The correlation is significant at $P<0.0001$. Data are provided in Supplementary Table S13.

et al., 2018). This information provides the context within which the breeding of maize hybrids has taken place.

Breeding has increased the generation of florets

The hybrids used here confirmed that breeding has significantly increased grain yield (Fig. 2A; $P<0.0001$) and that this effect has largely been due to increases in the total number of kernels (Fig. 2B, C; $P<0.001$ and $P<0.0001$, respectively) (Russell, 1984; Tollenaar and Wu, 1999; Echarte *et al.*, 2000; Tollenaar and Lee, 2002; Borrás and Vitantonio-Mazzini, 2018). A more detailed analysis revealed that breeding has increased the number of florets in the upper ear (Fig. 2D, Supplementary Fig. S1), the number of silked florets (Fig. 2E), and the number of kernels at maturity (Fig. 2F), all of which are defined during the 30-d critical period centred around silking. The changes in floret number indicated that breeding has modified early reproductive development, an effect that (to the best of our knowledge) has not been reported before. The proportion of silked florets slightly but significantly decreased with YOR (Fig. 2E, inset) and the rate of abortion did not change (Fig. 2F, inset). High PP had no effects on floret number and silking whilst producing an expected increase in abortion rate ($P<0.02$; see also Otegui, 1997, Cagnola *et al.*, 2018). Taken together, these results indicated that modern hybrids produce more florets and most of them persist through the critical period to maturity. Therefore, in subsequent experiments we focused on the critical period to investigate the processes that support the additional florets.

Breeding has enhanced ear growth, not vegetative shoot growth, during the critical period

Breeding has enhanced the ear growth rate during the critical period (Fig. 2G) (Echarte *et al.*, 2000; Luque *et al.*, 2006), and was directly related to kernel number (Fig. 2H). However, breeding had no significant effects on vegetative growth (leaves plus stem) during the critical period (Fig. 2I). This observation is significant because it indicates that the ear growth response does not reflect a change in overall growth capacity. There are cases in which breeding and selection for yield has resulted in maize plants of shorter stature (Russell, 1984; Meghji *et al.*, 1984), which might conceivably help divert resources to the ear; however, we did not observed any trend in plant stature in the hybrids used here (Supplementary Fig. S2). Furthermore, we did not observe changes in the leaf area of the whole canopy, in the stratum that contains the ear, or in the ear leaf itself in response to YOR (Supplementary Fig. S3). We therefore investigated the occurrence of localised changes in carbon balance in leaves placed around the ear as a potential alternative source to support the enhanced ear growth.

Breeding has increased the net CO_2 exchange rate of the ear leaf

We used a portable system to measure the rate of net CO_2 exchange of the leaf subtending the ear in field-grown maize crops. In different experiments, we either produced response curves to PAR and interpolated CO_2 exchange rates to the PAR incident on the ear leaf (Fig. 3A, Supplementary Fig. S4) or set the light source of the system to the PAR recorded simultaneously at the ear level in the field (Fig. 3B). Both approaches yielded similar results, with the rate of CO_2 exchange increasing with the YOR (net CO_2 exchange, $P<0.001$; normalised CO_2 exchange $P<0.02$). Differences in net CO_2 exchange could result from changes in PAR intercepted by the leaves, changes in leaf photosynthetic capacity, and/or changes in leaf respiration, and hence we next explored these three possibilities.

Breeding has increased light interception by the ear stratum of the canopy

Two field experiments showed that the PAR reaching the ear stratum increased with YOR (Fig. 4A, B, $P<0.0001$; 4C, $P<0.012$; see also Perez *et al.*, 2019, and Zhao *et al.*, 2015). These observations indicated that artificial selection for yield at high PP indirectly increased PAR penetration at ear height. PAR values recorded in the field significantly decreased with lower positions within the canopy and higher PP, and increased with the YOR (Fig. 4A, B). Notably, height within the canopy, PP, and YOR showed a significant interaction ($P<0.0001$). This reflects the observation that the canopies of modern genotypes grown at high PP received more PAR in the middle stratum (i.e. around the site of ear insertion) than those of older cultivars, whilst there were no significant effects of YOR on the upper and lower strata (Fig. 4B). More specifically, at high PP, the difference in PAR between the middle and lower strata significantly increased with YOR ($P=0.0002$), indicating that the stratum around the ear received and absorbed more PAR, rather than simply allowing more light to reach the lower stratum.

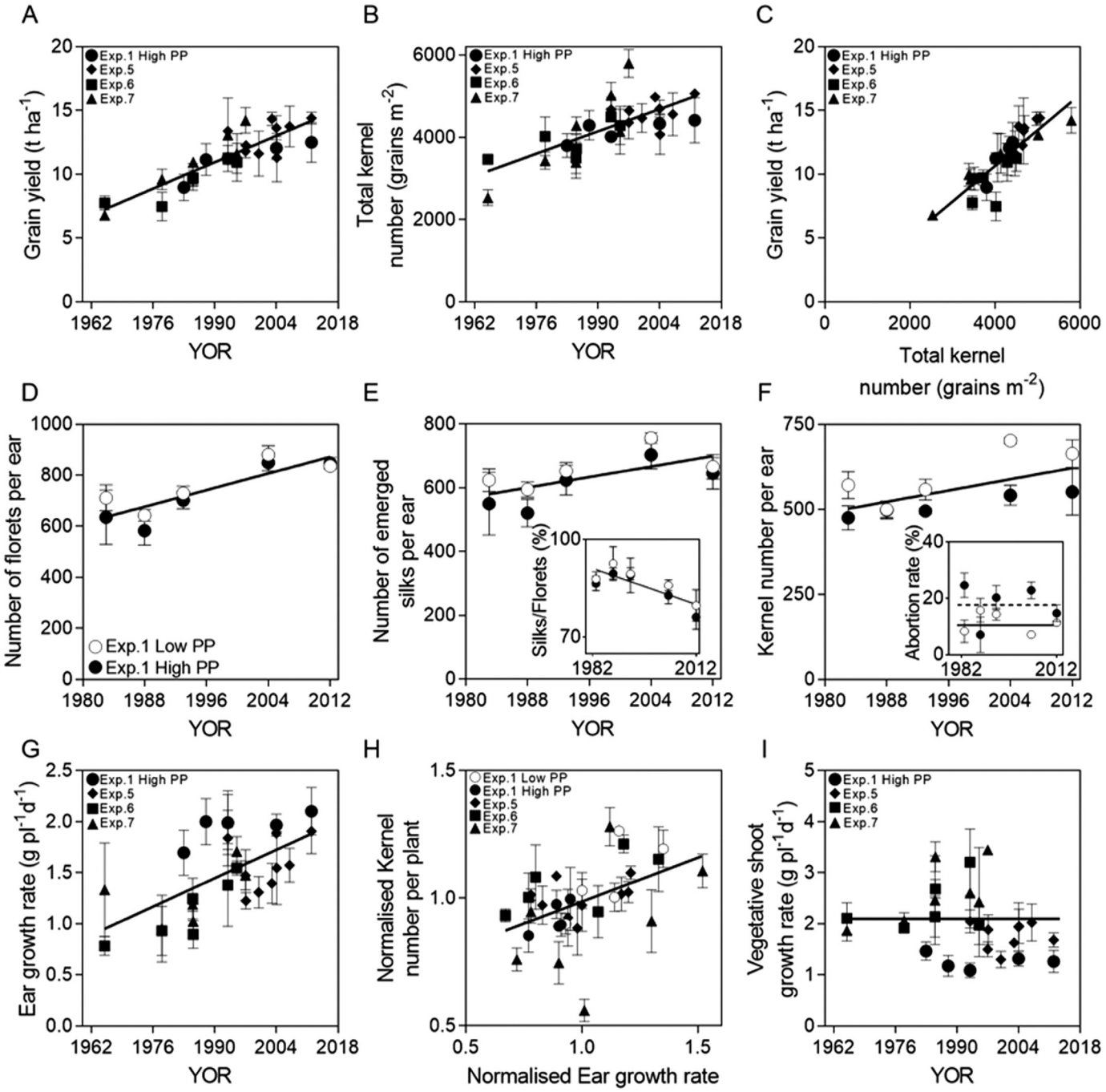

Fig. 2. Maize yield components affected by breeding. Correlations between the year of release (YOR) of the hybrid in Argentina versus crop grain yield (A) and kernel number per unit soil area (B), floret number (D), silked floret number (E), and kernels per plant (F), ear growth rate (G) and shoot growth rate (I) during the critical 30-d period centred on silking. Correlations between grain yield and kernel number (C) and between kernel number and ear growth during the critical period (H) are also shown. The insets in (E, F) show the proportions of silked florets and the abortion rate, respectively. In (H), the values have been normalised dividing by the mean rate of all the hybrids of each plot to minimise the effects of experiments and PP, and to facilitate visualisation of the general trend. Plants were grown at high plant population densities (PP, 8–9 plants m^{-2}), except for (D–F, H) where low PP condition (4 plants m^{-2}) were also included (open symbols). Data are means (±SE) from three replicate plots in the different experiments as indicated (see Methods for details). Lines indicate that the slopes are significantly different from zero ($P<0.05$), unless they are horizontal.

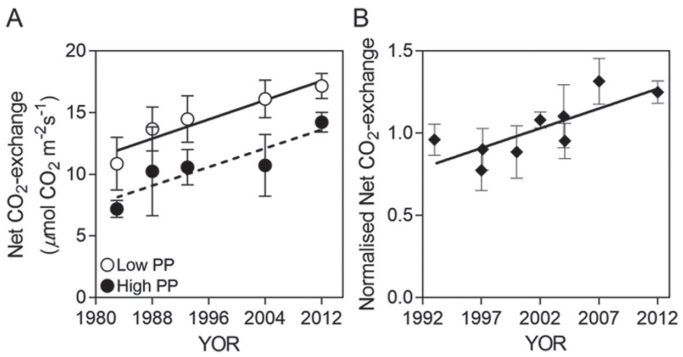

Fig. 3. Breeding has increased the rate of net CO_2 exchange of the ear leaf in maize during the critical 30-d period centred on silking. Correlations are shown between gas exchange and the year of release (YOR) of the hybrid in Argentina. (A) Plants were grown at either high plant population (PP) density (8–9 plants m^{-2}) or low PP (4 plants m^{-2}). Data are means (±SE) of three replicate plots from experiment 1 (see Methods for details). (B) The rates of gas exchange have been normalised by dividing by the mean rate of all the hybrids of each plot to minimise the effects of measurement time. Measurements were obtained under PAR levels observed within the ear strata in the field. All the slopes are significantly different from zero (P<0.05).

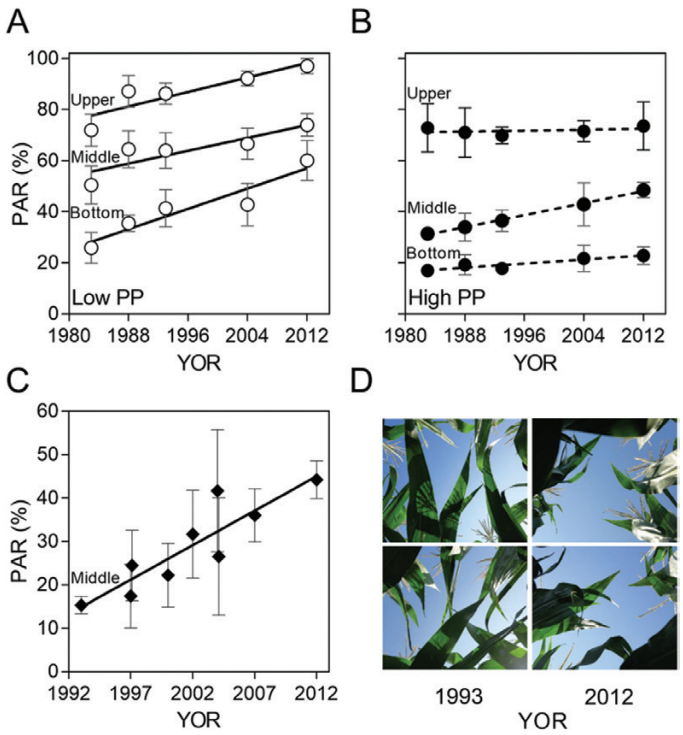

Fig. 4. Breeding has increased the penetration of photosynthetically active radiation (PAR) within the canopy of maize crops. PAR, expressed as a percentage of the value measured above the canopy, is plotted against the year of release (YOR) of the hybrid in Argentina. Plants were grown at either high plant population (PP) density (8–9 plants m^{-2}) or at low PP (4 plants m^{-2}). Data are from experiment 1 (A, B) and from experiment 5 (C) (see Methods for details), and are presented as means (±SE). All correlations are significant at P<0.05. (D) Representative images from experiment 5 taken within the canopies of hybrids of two different YOR, facing upwards at the height of the ear.

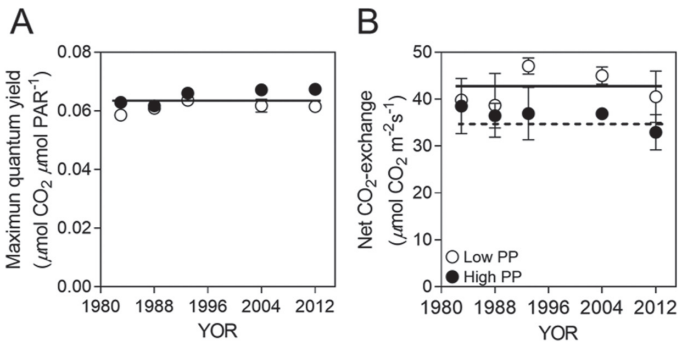

Fig. 5. Breeding has not affected the intrinsic photosynthetic capacity of the ear leaf in maize hybrids. (A) The apparent maximum quantum yield of CO_2 assimilation and (B) the net CO_2 exchange at a PAR of 2000 μmol m^{-2} s^{-1} are plotted against the year of release (YOR) of the hybrids in Argentina. Crops were grown at either high plant population (PP) density (8 plants m^{-2}) or low PP (4 plants m^{-2}). Data are means (±SE) of three replicate plots in experiment 1 (see Methods for details). The horizontal lines indicate that the slopes are not significant, but in (B) there was a significant effect of PP on the relationship (P<0.05).

Breeding has reduced ear leaf respiration

The higher rates of net CO_2 exchange of the ear leaf (Fig. 3) could have resulted from the observed differences in PAR reaching the ear-leaf stratum (Fig. 4), from differences in apparent maximum quantum yield of CO_2 assimilation (Pignon *et al.*, 2017), from differences in the capacity for light-saturated photosynthesis, and/or from differences in respiration. In order to examine this, we measured net CO_2 exchange rate as a function of PAR provided by the built-in light source of the portable gas-exchange system. Measurements were performed on the ear leaf of plants of five hybrids grown at high or low PP (Supplementary Fig. S4). The apparent maximum quantum yield of CO_2 assimilation (Pignon *et al.*, 2017) was not affected by PP or YOR (Fig. 5A). Net CO_2 exchange rate at a PAR of 2000 μmol m^{-2} s^{-1} (close to the maximum attainable midday PAR at the latitude of the experiments) decreased with high PP (P<0.001; Fig. 5B), indicating that mutual shading reduced the photosynthetic potential of the ear leaf (see also Dwyer *et al.*, 1991; Pignon *et al.*, 2017; Yabiku *et al.*, 2020) but showed no correlation with YOR. The net CO_2 exchange rate at a PAR of 0 μmol m^{-2} s^{-1} equates to the rate of respiration, and it was notable that this decreased at high PP (Fig. 6A; see also Pignon *et al.*, 2017; Yabiku *et al.*, 2020) and with YOR, and an interaction was observed (PP×YOR, P=0.012). We confirmed the trend between respiration and YOR in a set of experiments with plants grown in pots (Fig. 6B; P<0.025). As a result of the effect on respiration, the net CO_2 exchange rate at limiting PAR increased with YOR (300 μmol m^{-2} s^{-1}, P<0.0001; Supplementary Fig. S4F). Stomatal conductance was not affected by YOR or PP, and higher rates of net exchange resulted in lower internal concentrations of CO_2 (Supplementary Fig. S5).

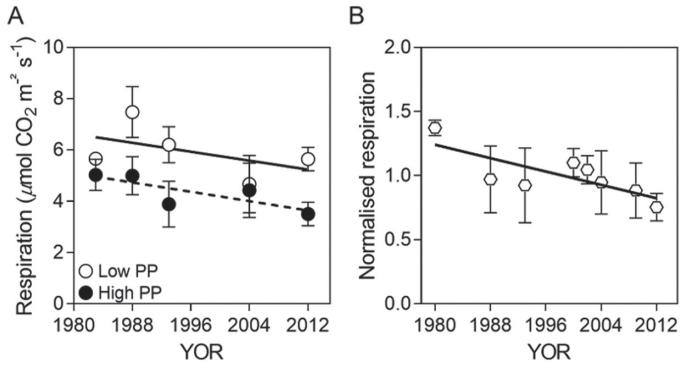

Fig. 6. Breeding has reduced the rate of respiration of the ear leaf in maize. Correlations are shown between the year of release (YOR) of the hybrids in Argentina and the rate of net CO_2 exchange at 0 μmol m^{-2} s^{-1} PAR measured at midday. In (A) plants were grown at either high plant population (PP) density (8 plants m^{-2}) or low PP (4 plants m^{-2}) or high PP () whilst in (B) plants were grown individually in pots. Data are means (±SE) of three replicate plots from experiment 1 (A) or experiments 2, 3, and 4 (B) (see Methods for details). The data in (B) are normalised by dividing by the mean of all the hybrids in each experiment (note that whilst the plants within an experiment were measured at the same temperature, it was different between experiments; Supplementary Table S2B). All correlations are significant at $P<0.05$.

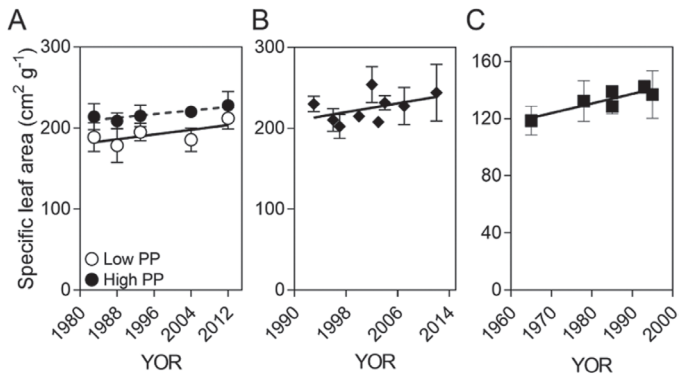

Fig. 7. Breeding has increased the specific leaf area of the ear leaf in maize. Correlations are shown between specific leaf area and year of release (YOR) of hybrids in Argentina. Plants were grown at either high plant population (PP) density (8–9 plants m^{-2}, closed symbols) or at low PP (4 plants m^{-2}, open symbols). Data are means (±SE) of three replicate plots from experiments 1 (A), 5 (B), and 6 (C) (see Methods for details). All correlations are significant at $P<0.05$.

Breeding has increased specific leaf area of the ear leaf

A priori, an increased leaf area per unit dry weight (specific leaf area, SLA) and/or a reduced chlorophyll content per unit area could account for the lower rates of respiration per unit leaf area of the ear leaf. The SLA of the ear leaf increased with the YOR in the three field experiments where we examined this trait (Fig. 7A, YOR×PP, $P=0.0011$; 7B, $P=0.03$; 7C, $P=0.026$). We observed a negative correlation between SLA and leaf respiration rate for the ear leaf across different hybrids and PPs (Supplementary Fig. S6, $P<0.002$). Chlorophyll content per unit area in the ear leaf increased with the YOR in the plants grown at low PP (Supplementary Fig. S7, $P<0.003$) but remained unaffected in plants grown at high PP. Therefore, breeding effects on SLA and not on chlorophyll have contributed to the changes in respiration with YOR. The effect of breeding on SLA was selective for the ear leaf stratum and was not observed for leaves located at either higher or lower positions in the canopy (Supplementary Fig. S8). Changes in SLA tend to have little impact on leaf absorbance per unit area (Evans and Poorter, 2001), and we did not observe a penalty for higher SLA on CO_2 uptake at high PAR (Fig. 5B) or chlorophyll content (Supplementary Fig. S7B) in modern hybrids.

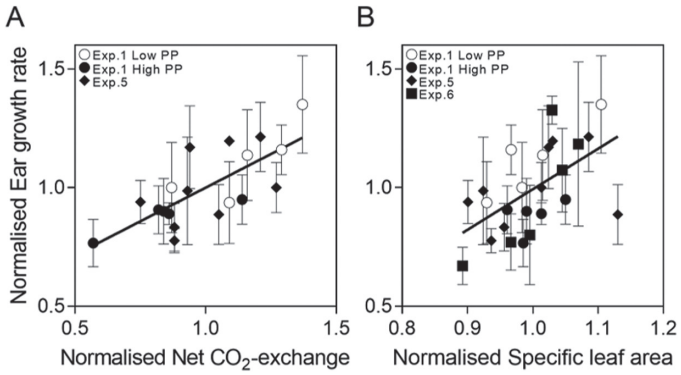

Fig. 8. Ear growth in maize during the critical 30-d period centred on silking correlates with net CO_2 exchange rate (A) and specific leaf area (B) of the ear leaf. Plants were grown at either high plant population (PP) density (8–9 plants m^{-2}) or at low PP (4 plants m^{-2}). The data have been normalised by dividing by the mean of all the hybrids in each experiment and PP to aid visualisation of the general trend. Data are means (±SE) of three replicate plots from experiments 1, 5, and 6 (see Methods for details). The correlations are both significant at $P<0.05$.

Ear leaf carbon balance is related to ear growth

Breeding has resulted in increases in ear-related traits, such as ear growth (Fig. 2G), and in leaf-related traits, such as net CO_2 exchange rate (Fig. 3) and SLA (Fig. 7). We found that accumulation of ear dry matter for different hybrids during the critical period directly related to ear-leaf net CO_2 exchange rate and to SLA (Fig. 8).

Discussion

The results presented here demonstrate that traditional breeding in maize based on grain yield has had the following three effects. First, it has increased the number of kernels per plant by elevating the number of florets (Fig. 2D) without enhancing floret silking rates or reducing abortion rates (Fig. 2E, F), at least in the absence of water or mineral shortages. Second, it has enhanced ear growth without associated changes in vegetative shoot growth (Fig. 2G, I), thus placing the focus on local processes in the region of the ear itself. And third, it

has enhanced the net CO_2 exchange of the ear leaf during the 30-d critical period centred on silking (Fig. 3) thanks to improved light interception (Fig. 4B) and reduced respiration rates (Fig. 6).

Although the effects of breeding on the number of kernels at harvest is well established (Echarte *et al.*, 2000; Tollenaar and Lee, 2002; Luque *et al.*, 2006; Ciancio *et al.*, 2016; Borrás and Vitantonio-Mazzini, 2018), our results place the origin of this effect at the very early stages of floret development, not at silking or kernel abortion (Fig. 2D–F). Breeding has not increased floret number in Ontario maize hybrids (Gonzalez *et al.*, 2021); however, compared to Argentinean hybrids, which are sink-limited (Borrás *et al.*, 2004), the Ontario hybrids have a shorter cycle and are source-limited.

Although the greater light penetration within crops of modern maize hybrids is well established (Ma *et al.*, 2014; Zhao *et al.*, 2015; Perez *et al.*, 2019), whether more PAR is actually intercepted during the critical period has remained unclear. Based on a three-dimensional model of plant architecture for a large set of European hybrids, Perez *et al.* (2019) have proposed that there is more efficient PAR interception by the ear stratum of modern genotypes; however, these light simulations were based on a virtual canopy that did not integrate plant–plant interactions and architectural plasticity, and hence the conclusions require experimental validation. Zhao *et al.* (2015) observed that greater PAR interception by the ear stratum in two hybrids released in the 1990s compared to two released in the 1970s did not occur until late during the tasselling-to-maturity phase, leaving uncertainty about the critical period. Here, we have shown that under high plant populations (PPs) the ear stratum of modern hybrids receives more PAR, which is effectively absorbed and does not reach the bottom stratum during the critical period (note the different slopes for the middle and bottom strata in Fig. 4B). We therefore conclude that breeding has improved PAR interception by the ear leaf. Hence, it is important that breeding programs evaluate traits such as PAR distribution so that their potential benefits are not inadvertently lost (Supplementary Fig. S9).

In crops grown at commercial PPs, shaded leaves may approach saturation at relatively low PAR values (Yabiku *et al.*, 2020), raising the question as to whether the ear leaf could take advantage of extra PAR interception. Here, we showed that in commercial crops the average PAR levels reaching the ear leaf are limiting for photosynthesis even at midday, as the increased PAR interception of the ear-leaf stratum with the year of release (YOR) (Fig. 4) effectively enhanced net CO_2 exchange of the ear leaf of modern hybrids (Fig. 3). Taken together, our results indicate that as a result of breeding, the ear leaf receives and intercepts more PAR, and uses this energy for photosynthesis.

Previous studies have reported elevated rates of leaf net CO_2 exchange at high PAR (2000 μmol m^{-2} s^{-1}) in modern hybrids during the post-silking period (Dwyer and Tollenaar, 1989; Dwyer *et al.*, 1991; Earl and Tollenaar, 1999; Richards, 2000; Lee and Tollenaar, 2007; Li *et al.*, 2019). In contrast, here we show that breeding has not affected net CO_2 exchange of the ear leaf at high PAR during the critical period (Fig. 5), indicating that fundamental differences in carbon balance exist between this period and kernel filling.

The reduced rate of respiration per unit area of the ear leaf (Fig. 6) could be accounted for by a concomitant increase in the specific leaf area (SLA; Fig. 7, Supplementary Fig. S6), i.e. a lower investment of dry matter per unit leaf area. A higher SLA at the whole-canopy level may have positive, neutral, or negative consequences for yield (Richards, 2000; Hund *et al.*, 2005; Trachsel *et al.*, 2010; Chen *et al.*, 2017; Gilardelli *et al.*, 2018). It is therefore interesting to note that breeding effects on SLA were localised in the ear-leaf stratum (Fig. 7, Supplementary Fig. S8). Genetic variation in SLA among maize hybrids is primarily caused by leaf thickness (Chen *et al.*, 2017), and in maize this is a trait under the control of phytochrome B (Wies *et al.*, 2019), a key sensory light receptor in the perception of PP (Casal, 2013).

Breeding programs have used steadily increasing PPs for the selection of hybrids (Fig. 1). Increasing PP increases shade (i.e. it lowers PAR) at the stratum of the canopy that contains the ear (Fig. 4A, B). Maize leaves acclimate to low irradiances by increasing SLA (Danila *et al.*, 2019) and consequently reducing the rate of respiration (Bellasio and Griffiths, 2014; Danila *et al.*, 2019). Furthermore, increasing SLA and reducing respiration are traits strongly linked to shade tolerance in a wide range of species (Baltzer and Thomas, 2007; Valladares and Niinemets, 2008; Burton *et al.*, 2017). Therefore, artificial selection for yield at high PP (Fig. 1) has converged with natural selection under shaded environments.

Maize yield is known to be more limited by sink than by source (i.e. photosynthesis) (Borrás *et al.*, 2004), and hence the primary action of breeding and selection for grain yield has probably been the generation of more florets (Fig. 2D). However, there are several lines of evidence to suggest that breeding effects on ear-leaf net CO_2 exchange are important for the success of the additional florets of modern hybrids during the critical period, which is a time of high susceptibility to stress. First, under normal conditions at least half of the carbohydrates produced by the ear leaf go to ear tissues during ear expansion (Suwa *et al.*, 2010) and kernel filling (Moutot *et al.*, 1986), and the ear leaf is the main source of carbohydrates for the ear (Jasdanwala and Khan, 1988). Indeed, reducing the photosynthesis of the leaves around the ear by shading shortly after pollination lowers the accumulation of dry matter in the ear (Shen *et al.*, 2020). Furthermore, limited removal of upper leaves to improve light penetration within the canopy increases carbon allocation to the ear (Liu *et al.*, 2015). Second, there has been a small but significant decrease in the proportion of silked florets in modern hybrids (Fig. 2E, inset), indicating that they mutually compete for resources during the critical period,

which is particularly costly in terms of respiration (André *et al.*, 1978). Third, a previous study has shown that application of 22 cycles of mass selection for increased grain yield to an indigenous Mexican maize variety reduced respiration of the ear leaf at anthesis when compared to the original population (Sarquís *et al.*, 1998). Fourth, the increased SLA (Fig. 7) and the higher rates of net CO_2 exchange of the ear leaf (Fig. 3) that we observed directly related to the enhanced growth of the ear during the critical period (Fig. 8), which in turn related to total kernel number (Fig. 2H). Fifth, high PP reduces the production of photoassimilates particularly in the middle stratum of the canopy that contains the ear, and lowers the proportion of plant assimilates diverted towards the ear (Edmeades and Daynard, 1979). Artificial selection under high PP (Fig. 1) has specifically opposed these trends by increasing net CO_2 exchange of the ear leaf (Fig. 3) and the partitioning of aboveground dry matter towards the ear (Fig. 2). These observations are consistent with a model where breeding and selection for grain yield in maize have synergistically increased both the sink and the source to support it.

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Trend in the number of florets per ear versus year of release of maize hybrids.

Fig. S2. Trend in plant height versus year of release of maize hybrids.

Fig. S3. Trend in leaf area in different canopy strata versus year of release of maize hybrids.

Fig. S4. Responses of net CO_2 exchange to PAR in maize hybrids at low and high plant populations.

Fig. S5. Trend in stomatal conductance versus year of release of maize hybrids, and relationships between CO_2 internal concentration and net CO_2 exchange.

Fig. S6. Relationship between respiration and specific leaf area in maize hybrids.

Fig. S7. Chlorophyll contents in three different canopy strata in maize hybrids.

Fig. S8. Specific leaf area in the upper and bottom strata of maize hybrids grown at high and low plant populations.

Fig. S9. Analysis of PAR, net CO_2 exchange, and specific leaf area including additional hybrids that have not been widely accepted commercially.

Table S1. Descriptions of the hybrids used in this study.

Table S2. Details of the environmental conditions for the experiments.

Table S3. Curve parameters for the CO_2 exchange responses to PAR.

Table S4. Details of the model used to estimate aboveground growth rate.

Table S5. Chlorophyll calibration curves.

Table S6. Database for Experiment 1.

Table S7. Database for Experiments 2–4.

Table S8. Database for Experiment 5.

Table S9. Database for Experiment 6.

Table S10. Database for Experiment 7.

Table S11. Summary of the variables measured in each experiment.

Table S12. Significant variables in the regression analysis.

Table S13. Database for the historical trend in plant population used in yield trials.

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Author contributions

JIC, MP, DHR, ELP, FC, JIA, SFL, GAM, and MEO. performed the experiments; JIC, GAM, MEO, and JJC. designed the experiments; JIC, MP, JIA, MEO, and JJC. analysed the data; JIC and JJC conceived the project; JIC and JJC. wrote the paper with contributions from all the authors.

Data availability

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

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