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RESEARCH ARTICLE

Enhancing yield of modern maize (*Zea mays* L.) hybrids through optimization of population photosynthetic capacity and light-nitrogen use efficiency under high planting density

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Highlights

- Modern maize hybrids exhibit optimized population characteristics under high density, though the potential for further genetic enhancement narrows.
- Modern hybrids retain enhanced photosynthetic capacity under high density and during the mid-reproductive stage.
- Over successive breeding eras, maize photosynthetic nitrogen use efficiency under high-density conditions has increased significantly, driving progressive gains in grain yield over time.

Abstract

In maize production, the development of density-tolerant and lodging-resistant varieties has made dense planting an effective strategy for achieving high and stable yields, with superior hybrids serving as a prerequisite for successful high-density cultivation. However, the photosynthetic mechanisms underlying improved density tolerance in maize hybrids released across different eras in China remain unclear. This study investigates 40 years of breeding progress toward enhanced photosynthetic traits under varying planting densities and elucidates the physiological and ecological bases of improved density tolerance in maize hybrids. A three-year field experiment was conducted from 2019 to 2021 to compare eight major Chinese hybrids from four decadal cohorts under three planting densities: 45,000 (D1), 67,500 (D2), and 90,000 (D3) plants ha^{-1}. At high density (D3), modern hybrids exhibited a more optimal canopy architecture and superior leaf photosynthetic performance compared to older hybrids, despite a slight reduction in specific leaf nitrogen. Notably, modern hybrids (2000s) were able to maintain higher net photosynthetic rates and photosynthetic nitrogen use efficiency (PNUE) at D3, resulting in the highest grain yield (GY), which was 118.47% greater than that of older hybrids (1970s). Leaf area duration after anthesis, total chlorophyll content, key photosynthetic enzyme activities, and maximum quantum efficiency of PSII photochemistry were all positively correlated with GY. Among these, PNUE showed the strongest correlation with grain yield and thus represents a key indicator for optimizing maize hybrids. Based on these findings, breeders should continue selecting hybrids under high-density and suboptimal conditions, focusing on optimizing population architecture and enhancing photosynthetic capacity while fine-tuning leaf nitrogen status to develop high-yielding, density-tolerant hybrids capable of sustaining long-term increases in maize grain yield.

Keywords: maize hybrids, planting density, photosynthetic characteristics, photosynthetic N use efficiency, grain yield

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1. Introduction

Maize (*Zea mays* L.) is the cereal crop with the highest total yield and the largest planting area in China, playing a critical role in ensuring national and global food security (Ma *et al.* 2022). Data indicate that China's maize production must increase by at least 30–40% over the next decade to meet rising population demands (Long 2019). However, current maize production faces significant challenges, including climate change, limited arable land, soil degradation, and increasing pressure from diseases and pests, all of which severely constrain planting density and limit the realization of maximum yield potential (Laurance *et al.* 2014; Zhao *et al.* 2020; Wang *et al.* 2021). Therefore, to satisfy growing population needs and diverse market demands, it is essential to ensure a continuous supply of high-quality maize hybrids to support global food security (Zhang R Y *et al.* 2018; Yan *et al.* 2019).

Improvements in maize yield are primarily driven by the interaction between genotype and environment, with the continuous development of superior hybrids being the most influential factor. Breeding advancements account for more than 50% of historical yield increases, reaching up to 70% in certain regions (Cardwell 1982; Zhang *et al.* 1998; Tollenaar and Lee 2002; Duvick 2005; Ci *et al.* 2011; Niu *et al.* 2013; Debruin *et al.* 2017). The progressive optimization of maize hybrids has enhanced optimal planting density, enabling higher plant populations without compromising productivity (Sarlangue *et al.* 2007). In studies of hybrid breeding, the concept of genetic gain has provided valuable insight into trait improvement trends over time, with yield genetic gain serving as a direct indicator of population-level yield enhancement (Russell 1991; Duvick 2005). Modern varieties exhibit higher canopy photosynthetic rates across varying densities, along with strong density tolerance, broad adaptability, adequate assimilate supply, large sink capacity, and high yield potential (Dong *et al.* 2000; Di Matteo *et al.* 2016). Nevertheless, Tokatlidis and Koutroubas (2004) demonstrated that the yield increase in modern hybrids stems largely from improved density tolerance rather than increased yield per individual plant, underscoring the importance of population-level performance in achieving high yields (Feng *et al.* 2014).

Planting density serves as a key interface between genotype and environment and represents one of the primary drivers of maize yield increases (Tollenaar and Wu 1999; Luo *et al.* 2023). High-density planting has become an effective strategy for achieving stable and high yields (Brekke *et al.* 2011; Assefa *et al.* 2016; Li *et al.* 2016). However, under excessively high densities, soil resources per plant decline, intensifying intraspecific competition (Antonietta *et al.* 2014). Additionally, shading within the canopy reduces light penetration, weakening illumination in the middle and lower leaf layers during later growth stages (Ren *et al.* 2017a). These constraints reduce individual plant photosynthesis and growth rates, ultimately limiting yield (Pagano *et al.* 2007; Zhang M *et al.* 2018). Consequently, breeders have increasingly selected genotypes based on population performance rather than individual plant superiority (Weiner *et al.* 2010). Through successive breeding cycles, the competitive ability of individual plants within populations has diminished, while tolerance to high density has significantly increased (Vandeleur and Gill 2004; Zhai *et al.* 2016). As a result, modern hybrids perform well at the population level under dense conditions and achieve higher yields (He *et al.* 2022). Since the 1950s, maize planting density in China has increased by an average of 500 plants ha^{-1} per decade to enhance productivity, although both yield levels and planting densities remain below those achieved in the United States during the same period (Luo *et al.* 2023). Dense planting alters plant architecture and developmental patterns, and the interaction between hybrid genetics and planting density is expected to become even more critical in future breeding programs (Luo *et al.* 2023). Adjusting planting density or selecting appropriate varieties can modify canopy structure and nitrogen distribution, aligning nitrogen allocation among leaves with available light resources. This coordination optimizes canopy light and nitrogen use efficiency, thereby improving photosynthetic efficiency (Yao *et al.* 2016; Song *et al.* 2017; Yu *et al.* 2022).

Studies on the interaction between maize hybrids from different eras and planting density have primarily focused on changes in plant architecture and yield-related traits (Ma *et al.* 2022; Jafari *et al.* 2024). In contrast, the responses of maize population characteristics and photosynthetic capacity under these interactions remain poorly documented, limiting understanding of historical shifts in photosynthetic performance. First, this study presents the first assessment of evolutionary trends in population characteristics and photosynthetic capacity of maize hybrids over a 40-year period under varying era-specific hybrids and density treatments. Second, we calculated and analyzed genetic gains for relevant traits to identify decade-wise improvement patterns in hybrid breeding. Thus, our experiment aimed to investigate: (1) the evolutionary patterns of maize population characteristics and photosynthetic capacity under interactions between era-specific hybrids and planting density; and (2) the coordinated optimization of light and nitrogen use efficiency in maize under such interactions. The objective is to elucidate the physiological and ecological mechanisms underlying improved density tolerance in maize hybrids, providing insights for breeding strategies and theoretical support for the ongoing renewal of maize hybrids in China.

2. Materials and methods

2.1. Experimental design

In this experiment, eight major maize hybrids widely promoted in China from 1970 to 2009 were selected (Appendix A). These hybrids represented the dominant cultivars in China's primary maize production regions during that period and thus reflect the varietal evolution across key maize-growing areas of the country.

The experimental design followed a split-plot arrangement. The main plots consisted of three planting density treatments: 45,000 plants ha^{-1} (D1), 67,500 plants ha^{-1} (D2), and 90,000 plants ha^{-1} (D3). The subplot factor comprised eight maize hybrids from different eras, resulting in 24 treatment combinations arranged in a randomized complete

block design with three replicates. Each plot measured approximately 30 m^2 (10 m×3 m) and contained five rows spaced 60 cm apart. Seeds were sown on June 15 in 2019 and 2020, and on June 21 in 2021. Three seeds were planted per hole at a depth of 5 cm, and seedlings were thinned to achieve the target density 15 days after sowing. At sowing, 750 kg ha^{-1} of compound fertilizer (N≥25%; P_2O_5≥5%; K_2O≥5%) was applied as basal dressing, and 150 kg ha^{-1} of urea was top-dressed at the jointing stage. All other management practices followed those typical of high-yield maize fields. Weeds and pests were controlled using chemical methods.

2.2. Site and meteorological data

The experiment was conducted at the Mazhuang Experimental Base, Shandong Agricultural University, Tai'an City (35°60′N, 117°01′E, 153 m a.s.l.) from 2019 to 2021. The site lies within the Huang-Huai-Hai Plain, China and has a temperate continental monsoon climate. The soil at the experimental field is classified as brown earth, characterized by a deep soil profile and high surface organic matter content, which is favorable for maize cultivation. Soil fertility parameters over the three-year trial are presented in Table 1. During the maize growing seasons from 2019 to 2021, the monthly mean air temperature at the site was 24.18°C, with total precipitation amounting to 648.03 mm (Appendix B). All meteorological data were provided by the Tai'an Meteorological Bureau.

2.3. Sampling and measurement

Leaf area At the 12th leaf stage (V12), silking stage (R1), and 15, 30, 45, and 60 days after silking (R1+15, R1+30, R1+45, R1+60), five uniformly growing plants with healthy foliage were selected per plot. The length and maximum width of all fully expanded leaves were measured using a meter stick. Leaf area was calculated using the following equation:

Single leaf area (m^2)=Length×Width×0.75 (Zhang *et al.* 2019)

Population leaf area (PLA)=Single plant leaf area×Planting density

LAI=Single leaf area×Number of plants per unit land area/Unit land area

Leaf area duration (LAD)=$(LAI_1+LAI_2)/2\times(T_2-T_1)$

where LAI_1 and LAI_2 are the leaf area indices measured at two consecutive time points; T_2–T_1 indicates the number of days between measurements.

Leaf angle (LA) and canopy light interception At R1, five consecutive maize plants were randomly selected in each plot. The angle between the stem and each of the three ear-associated leaves — ear leaf, upper leaf adjacent to the ear, and lower leaf below the ear — was measured, with three replications per treatment; mean values were calculated.

Photosynthetically active radiation (PAR) was measured using a SunScan canopy analyzer (SS1 SunScan, Delta-T Devices Ltd., UK) between 9:00 a.m. and 11:00 a.m. on clear, cloudless days at R1 and R1+30. Five measurement points were selected diagonally between rows within each plot and averaged. Incident PAR (IPAR) was recorded at the top of the canopy, while transmitted PAR (TPAR) was measured at 15 cm above the ground and at the ear level (midpoint of the ear leaf). Canopy light interception was calculated as:

Light interception rate (LIR) (%)=1–TPAR/Incident photosynthetically active radiation (IPAR)

Chlorophyll content (Chl) and photosynthesis key enzymatic activity Chl was determined following the method of Zhang (1986). At R1 and R1+30, three maize plants per treatment were sampled, and ear leaves were extracted using an acetone-ethanol (1:1, v/v) mixture. Extract absorbance was measured at 663 and 645 nm using a UV spectrophotometer (UV-2450, SHIMADZU, Japan), and chlorophyll concentrations were estimated as follows:

Chl *a*=$12.72OD_{663}$–$2.59OD_{645}$

Chl *b*=$22.88OD_{645}$–$4.67OD_{663}$

Chl (*a*+*b*)=Chl *a*+Chl *b*=$20.29OD_{645}$+$8.05OD_{663}$

For determination of key photosynthetic enzyme activities, three maize plants were sampled per plot at silking stage; ear leaves were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPC) activities were assayed using plant enzyme kits (Suzhou Grace Biotechnology Co., Ltd., Jiangsu Province, China) according to the manufacturer's instructions.

Net photosynthetic rate (P_n) and chlorophyll fluorescence parameters P_n of the ear leaf was measured using an open-flow portable photosynthesis system (CIRAS-3, PP Systems, Hansatech, UK) at R1 and R1+30 under clear weather conditions between 9:00 a.m. and 12:00 p.m. Light intensity, CO_2 concentration, and leaf temperature were maintained at 1,600 μmol m^{-2} s^{-1}, 400 μmol mol^{-1}, and 30°C, respectively. Measurements were taken from the mid-portion of healthy leaves; five leaves per treatment were assessed, and the mean value was recorded.

Chlorophyll fluorescence was measured on the same leaves used for gas exchange analysis, using a portable fluorometer (M-PEPC activities (PEA), Hansatech, UK). Measurements were conducted on cloudless days between 10:00 a.m. and 12:00 p.m. under incident photosynthetic photon flux density (PPFD) of approximately 1,600 μmol m^{-2} s^{-1}. Leaves were dark-adapted for 30 min prior to measurement. Initia fluorescence (F_o) was recorded, and maximum fluorescence (F_m) was induced by a saturating pulse of light. The maximum quantum efficiency of PSII photochemistry (F_v/F_m) in the dark-adapted state was calculated as $(F_m–F_o)/F_m$, following Demmig-Adams *et al.* (1996).

Specific leaf nitrogen (SLN) and photosynthetic nitrogen utilization efficiency (PNUE) Plants were harvested at V12, R1, R1+15, R1+30, R1+45, and R1+60 to determine dry matter accumulation. Five plants per treatment were collected and separated into leaves, stems, tassels, bracts, and female ears. All plant components were oven-dried at 105°C for 30 min to inactivate enzymes, then dried at 80°C for 3–5 days until constant weight. Dried samples were

Table 1 Nutrition content in test base in Mazhuang Town, Tai'an City, Shandong Province, China in 2019

Organic matter (g kg^{-1})	Total N (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
11.20	0.71	56.43	28.33	109.01

ground for nutrient analysis. Total leaf nitrogen content was determined after digestion with concentrated H_2SO_4-H_2O_2 using a continuous flow analyzer (AutoAnalyzer 3, SEAL Analytical GmbH, Norderstedt, GER). SLN was calculated as nitrogen content per unit leaf area (Mu *et al.* 2016). PNUE reflects the efficiency of nitrogen utilization in photosynthesis (Yu *et al.* 2022).

SLN (g m^{-2})=Leaf nitrogen content/Leaf area

PNUE (μmol CO_2 g^{-1} N s^{-1})=P_n/SLN

Grain yield (GY) At physiological maturity, all plants in a 9 m^2 central portion of each plot were harvested. The number of barren plants (lacking ears) was counted to compute the barren ear rate. Ear number per unit area and GY were recorded. Subsequently, 30 ears were randomly selected for assessment of ear traits. Kernel number per ear and 1,000-kernel weight were determined. For 1,000-kernel weight, grains were dried at (50±5)°C to constant weight with five replications per plot. Final grain weights were adjusted to a standard moisture content of 14%.

GY (kg ha^{-1})=Ears ha^{-1}×Kernels/Ear×Dry grain weight (g)/(100%–14%)

where grain weight refers to the average kernel mass derived from the 1,000-kernel weight (Yu *et al.* 2022).

Index definition Maize population characteristics measured in this study included PLA, LAD, LA, and canopy light interception. To visualize evolutionary trends in population traits among maize hybrids across decades, hybrids were grouped into three era intervals: era interval 1 (E1): 1970s–1980s, era interval 2 (E2): 1980s–1990s, and era interval 3 (E3): 1990s–2000s). Following Duvick (2005), genetic gains for each trait were computed within each era interval to identify periods of significant improvement. Additionally, total genetic gains for each trait over the entire 40-year period were calculated. The annual genetic gain (G) was estimated as:

G $year^{-1}$=(A2–A1)/Y

where A1 and A2 represent the trait values at the beginning and end of an era interval, respectively, and Y is the number of years in the era interval.

2.4. Statistical analyses

Data were processed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA), and statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). Analysis of variance (ANOVA) was conducted to evaluate treatment effects across the three years. Differences among treatments were assessed using the least significant difference (LSD) test at P<0.05. Graphs were generated using Origin 2021 (OriginLab Corp., Northampton, MA).

3. Results

3.1. Response of LAD and its genetic gain to plant density

Over the 3-year experiment, hybrids released in different decades exhibited significant differences in LAD across the three tested planting densities (D1, D2, and D3). Modern hybrids from the 2000s showed an 8.34% increase in pre-anthesis leaf area duration (PALAD), a 45.52% increase in post-anthesis leaf area duration (AALAD), and a 27.35% increase in total leaf area duration (TLAD) compared to older hybrids from the 1970s (Fig. 1, P<0.05). Concurrently, modern hybrids exhibited a significantly slower decline in PLA after anthesis than older hybrids, as confirmed by mixed-model ANOVA (F=6.31; P<0.05). The green leaf retention advantage of modern hybrids became increasingly apparent as the reproductive period progressed (Appendix C).

During the 3-year study, genetic gains in PLA between different era intervals varied significantly across reproductive stages. An absolute advantage in PLA genetic gains was observed at E1 before R1+30, whereas the largest gains occurred at E2 after R1+30 (P<0.05; Appendix D). The maximum total PLA genetic gains were recorded at R1+45 and R1+60 under all densities, with the highest values at D3 — averaging 441.55 and 724.25 m^2 ha^{-1} yr^{-1} over the 3 years. The pattern of LAD genetic gains across densities was consistent among era intervals, with TLAD genetic gains primarily driven by AALAD genetic gains (P<0.05; Appendix E). D2 E2 and D3 E2 achieved the greatest genetic gains in both AALAD and TLAD, averaging 5.37 and 6.59 m^2 d m^{-2} yr^{-1}, and 6.24 and 7.27 m^2 d m^{-2} yr^{-1}, respectively, over the 3 years.

3.2. Response of genetic gains of LA and population light interception to plant density

In response to varying planting densities, LA, canopy light interception rate (CLIR), and mid-upper layer light interception rate (MULIR) of hybrids from different decades displayed distinct performance patterns in 2019 and 2020. Both planting density and hybrid era, along with their interactions, had significant effects on these traits (P<0.05; Appendices F and G). LA decreased progressively with hybrid advancement across all densities. At D3, maize hybrids from the 2000s exhibited average reductions in leaf angle of 17.53% (*vs.* 1970s), 8.20% (*vs.* 1980s), and 9.02% (*vs.* 1990s) during the 2-year experiment. Under high-density conditions, modern hybrids had significantly lower CLIR than older hybrids at R1 and R1+30, while their mid upper layer light interception rate (MULIR) was significantly higher.

Over the 2-year experiment, genetic gains in LA and LIR differed significantly among era intervals at each density (P<0.05; Table 2). The greatest genetic gain in LA at each density occurred during E1, with 2-year average annual reductions of 1.38° (D1), 0.56° (D2), and 0.44° (D3) yr^{-1}. CLIR showed maximum genetic gains at E3, with 2-year average annual reductions of 0.225 and 0.384% under D3 at R1 and R1+30, respectively. For MULIR, the largest genetic gain was observed at D2 E1, with 2-year average reductions of 0.241 and 0.389% yr^{-1} at R1 and R1+30.

3.3. Response of the chlorophyll content and photosynthesis key enzymatic activity (PKEA) of ear leaf to plant density

Chl *a*, Chl *b*, and total chlorophyll content (TCC=Chl *a*+*b*) increased progressively with hybrid release era during the 2-year trial (P<0.05; Fig. 2; Appendix H). At D3, TCC in 2000s hybrids was on average 23.72, 16.19, and 7.33%

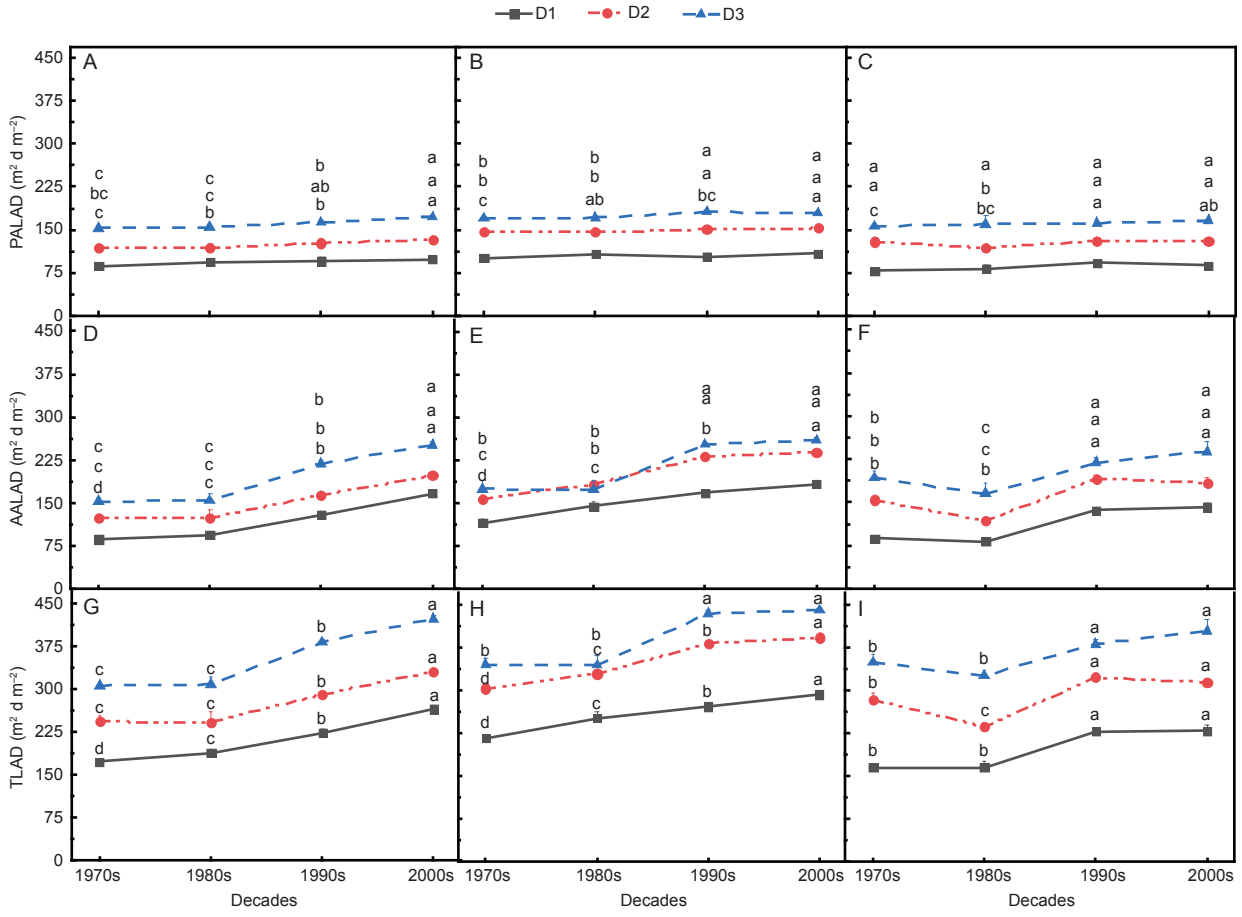


Fig. 1 Changes trend of leaf area duration (LAD) of maize hybrids at three planting densities in 2019, 2020 and 2021. A–C are pre-anthesis leaf area duration (PALAD) in 2019–2021. D–F are post-anthesis leaf area duration (AALAD) in 2019–2021. G–I are the total leaf area duration (TLAD) in 2019, 2020, and 2021. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. Bars mean SE (n=3). Values in the same character were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05.

higher than that of 1970s, 1980s, and 1990s hybrids, respectively. Similarly, Chl *a* increased by 25.40, 17.07, and 8.17%, and Chl *b* increased by 19.19, 13.97, and 4.75%. Additionally, from R1 to R1+30 at D3, TCC declined by 27.45% (1970s), 21.46% (1980s), 18.83% (1990s), and 19.16% (2000s) on average across 2019 and 2020, indicating that modern hybrids maintained higher chlorophyll levels during late reproduction.

Significant differences in PKEA among era-specific hybrids were observed under density treatments in 2020 (P<0.05; Fig. 3). Modern hybrids demonstrated a clear advantage in PKEA under high density: Rubisco activity (RA) in 2000s hybrids increased by 12.52, 19.08, and 23.64% compared to 1970s, 1980s, and 1990s hybrids at D3, respectively, while PEPC activity (PEA) increased by 14.59, 33.03, and 53.18%.

3.4. Response of chlorophyll fluorescence parameters and P_n to plant density

During the 2-year experiment, significant differences were observed in F_v/F_m, of ear leaves among hybrids from different eras under the three planting densities at R1 and R1+30 (P<0.05; Fig. 4; Appendix H). Increasing planting density from D1 to D3 at R1 reduced F_v/F_m by 3.66, 3.54, 2.96, and 2.57% across the four hybrid eras on average in 2020 and 2021. At D3, 2000s hybrids exhibited ear leaf F_v/F_m values that were 3.66, 3.33, and 2.00% higher than those of 1970s, 1980s, and 1990s hybrids, respectively, over the 2-year period.

Hybrids from different eras showed significant variation in ear leaf P_n under varying densities during the 2-year experiment (P<0.05; Fig. 5; Appendix H). Increasing density from D1 to D3 at R1 reduced P_n by averages of 35.00, 38.11, 26.68, and 18.90% across the four decades in 2020 and 2021. Notably, at D3 and R1, P_n in 2000s hybrids increased by 47.51, 46.67, and 17.29% compared to 1970s, 1980s, and 1990s hybrids, respectively. This indicates that modern hybrids better maintain photosynthetic capacity under high-density conditions.

3.5. Response of SLN and PNUE to plant density

Significant differences in SLN were observed among hybrids from different decades at each density in the 2-year trial, with both hybrid era and density, as well as their interaction, exerting significant effects (P<0.05; Appendix I). SLN decreased with increasing density, and 2000s hybrids generally exhibited lower SLN than older hybrids across reproductive stages. However, modern hybrids maintained

Table 2 Changes of genetic gains of leaf angle (LA) and light interception rate (LIR) of maize hybrids at three planting densities in 2019 and 2020

Density[1)]	Era interval[2)]	Genetic gain of LA (° yr^{-1})	Genetic gain of LIR (% yr^{-1})[3)]			
			R1		R1+30	
			Canopy	Middle and upper layers	Canopy	Middle and upper layers
2019						
D1	E1	–0.86 b	–0.238 a	0.242 a	–0.207 a	0.192 a
	E2	–0.11 a	–0.036 a	0.080 a	–0.043 a	0.068 a
	E3	–0.09 a	–0.314 a	0.130 a	–0.491 a	0.143 a
	Total	–0.36	–0.196	0.151	–0.218	0.134
D2	E1	–0.49 b	–0.127 a	0.246 a	0.013 a	0.413 a
	E2	–0.16 a	–0.071 a	0.166 ab	0.103 a	0.280 ab
	E3	–0.14 a	–0.170 a	0.056 b	–0.217 b	0.092 b
	Total	–0.27	–0.123	0.156	–0.034	0.262
D3	E1	0.01 a	–0.038 a	0.242 a	–0.223 a	0.177 a
	E2	–0.08 a	–0.165 ab	–0.020 b	–0.069 a	0.224 a
	E3	–0.11 a	–0.333 b	0.044 ab	–0.574 b	0.217 a
	Total	–0.06	–0.179	0.111	–0.278	0.206
2020						
D1	E1	–1.89 c	–0.201 a	0.002 a	–0.065 a	0.050 a
	E2	0.03 a	–0.098 a	0.017 a	–0.120 a	0.110 a
	E3	–0.33 b	–0.268 a	0.056 a	–0.293 a	0.277 a
	Total	–0.73	–0.189	0.025	–0.159	0.146
D2	E1	–0.63 c	–0.082 a	0.236 a	–0.118 a	0.364 a
	E2	–0.02 a	–0.099 a	0.136 ab	–0.380 ab	0.242 ab
	E3	–0.30 b	–0.225 a	0.023 b	–0.702 b	0.061 b
	Total	–0.32	–0.136	0.132	–0.400	0.222
D3	E1	–0.89 c	–0.108 a	0.056 b	–0.120 a	0.126 a
	E2	0.18 a	–0.031 a	0.115 a	–0.075 a	0.006 b
	E3	–0.49 b	–0.116 a	0.052 b	–0.193 a	0.133 a
	Total	–0.40	–0.085	0.074	–0.129	0.088
Analysis of variance (ANOVA)						
Year (Y)		***	***	*	***	NS
Density (Den)		***	***	*	**	NS
Era interval (E)		***	*	***	***	NS
Y×Den		***	***	NS	**	**
Y×E		***	NS	*	*	*
Den×E		***	NS	NS	NS	*
Y×Den×E		***	NS	NS	NS	**

[1)] D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively.
[2)] E1–E3, era interval 1, 1970s–1980s; era interval 2, 1980s–1990s; era interval 3, 1990s–2000s.
[3)] R1, silking stage; R1+30, 20 days after silking.
Values in the same character were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05. NS, *, ** and *** showed no significant difference and significant differences at 0.05, 0.01 and 0.001 levels, respectively.

SLN more effectively during mid-reproduction under high density. Specifically, SLN decreased by averages of 12.60, 10.97, 12.02, and 11.27% at R1, and by 17.15, 24.70, 17.74, and 12.19% at R1+30 across the four eras. Some older hybrids exhibited unusually high SLN (>3 g m^{-2}) at R1+60 due to extremely small green leaf area (<0.1 m^2), though nitrogen measurements included contributions from senesced tissue.

To assess the efficiency of leaf nitrogen use in photosynthesis, photosynthetic nitrogen use efficiency (PNUE) was calculated. PNUE differed significantly among era hybrids at R1 and R1+30 under all densities in the 2-year trial (P<0.05; Fig. 6; Appendix H). At D3, 2000s hybrids exhibited average increases in PNUE of 64.36, 56.56, and 18.87% at R1, and 70.99, 42.50, and 36.82% at R1+30 compared to 1970s, 1980s, and 1990s hybrids, respectively. Furthermore, modern hybrids exhibited smaller declines in PNUE under high density and maintained higher PNUE during later reproductive stages. The largest increase in PNUE between consecutive eras occurred from the 1990s to 2000s at D3, with a mean increase of 31.82% over the 2-year trial.

3.6. Response of genetic gains in GY to plant density

Among all treatments in the 3-year trial, 2000s hybrids at D3 achieved the highest GY, averaging 11.71 t ha^{-1}, while 1970s hybrids at D3 yielded the least, averaging only 5.36 t ha^{-1} (Appendix J). Genetic gains in GY varied by era interval and density: E1 showed the maximum gain under D1 (0.137 t ha^{-1} yr^{-1} over 3 years), whereas E2 produced the largest gain under D3 (0.312 t ha^{-1} yr^{-1}) (Fig. 7). Notably, total genetic

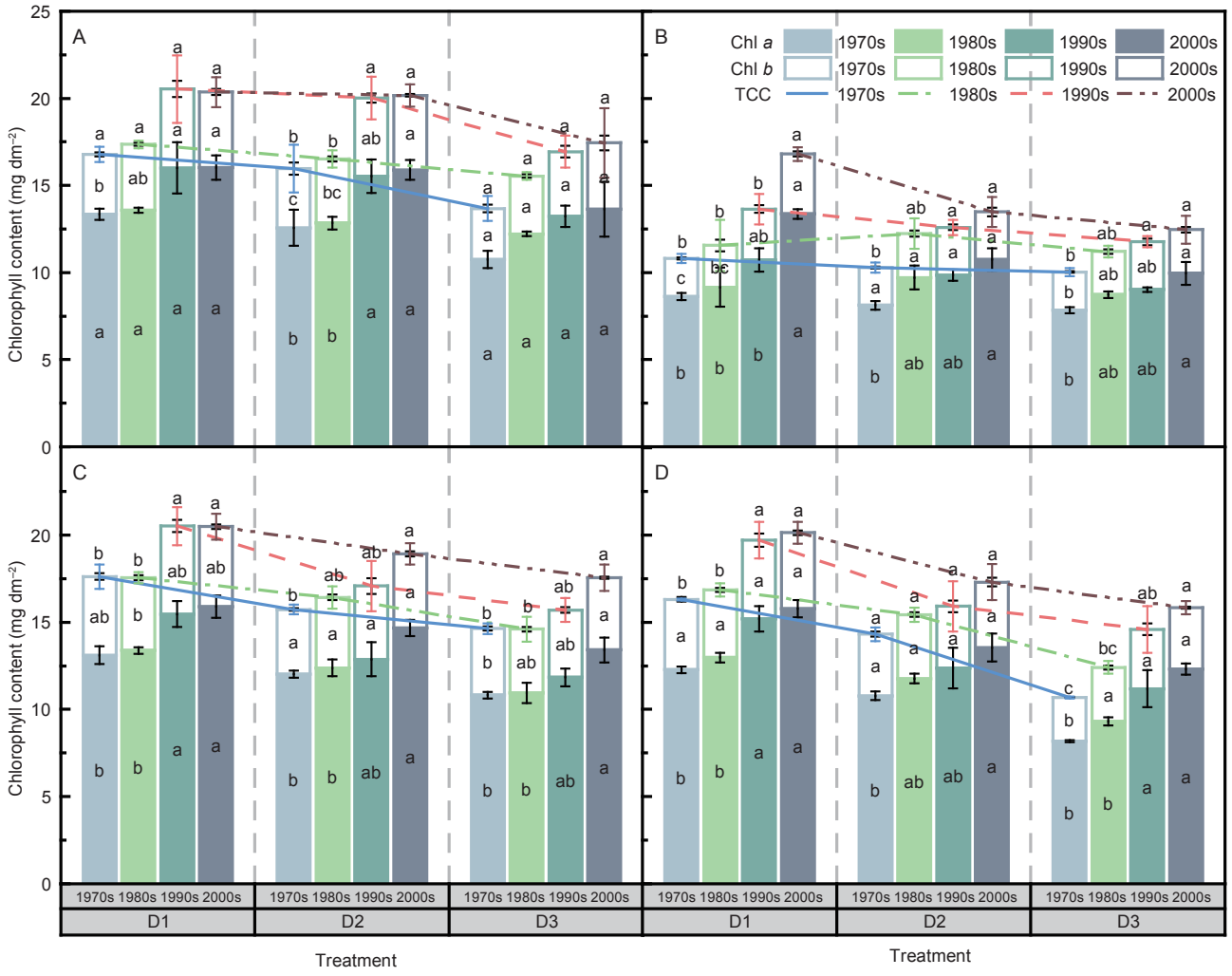


Fig. 2 Changes of chlorophyll (Chl) content of ear leaf of maize hybrids at three planting densities in 2019 and 2020. A and C are Chl *a* contents of silking stage (R1) in 2019 and 2020, B and D are Chl *a* contents of 20 days after silking (R1+30) in 2019 and 2020. Total chlorophyll content (TCC) was the total amount of Chl *a* and Chl *b* contents. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. Bars mean SE (n=3). Values in the same character were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05.

gain in GY under D3 was significantly greater than under D1 or D2 over the 40-year breeding period, indicating that high yield under high-density conditions were viewed more favorably. Overall, 2000s maize hybrids demonstrate greater tolerance to dense planting and are more likely to achieve high yields under such conditions compared to older hybrids.

3.7. Differences and relationships among hybrids and density

Principal component analysis (PCA) summarized variation among hybrids of different eras and densities based on 14 photosynthesis-related traits. The first two principal components explained 81.6% of the total variation in functional traits (P<0.05; Fig. 8-A). Additionally, correlation analyses were conducted among grain yield, population characteristics, and photosynthetic functional indicators (Fig. 8-B). GY was significantly positively correlated with AALAD, MULIR, TCC, PKEA, F_v/F_m, and PNUE, but showed no significant correlation with TLAD, P_n, or SLN. Moreover, ear leaf P_n was significantly positively correlated with TCC, PKEA, F_v/F_m, and PNUE, and significantly negatively correlated with PLA and CLIR.

4. Discussion

4.1. Modern hybrids had optimized population characteristics under high density

Optimal canopy structure is essential for achieving high yields under high-density conditions, with leaf area index and LAD serving as key indicators of crop canopy performance (Liu *et al.* 2017; Yang *et al.* 2022). In this experiment, PLA and LAD increased progressively with the advancement of modern maize hybrids, and the total genetic gain in these traits was particularly significant at high planting densities (Fig. 1; Appendix E). This improvement is primarily attributed to modern hybrids having longer spike leaves, significantly larger individual leaf areas, and higher leaf area per plant (Ma *et al.* 2014). Additionally, modern hybrids exhibit a strong "stay-green" capacity, characterized by faster leaf development and

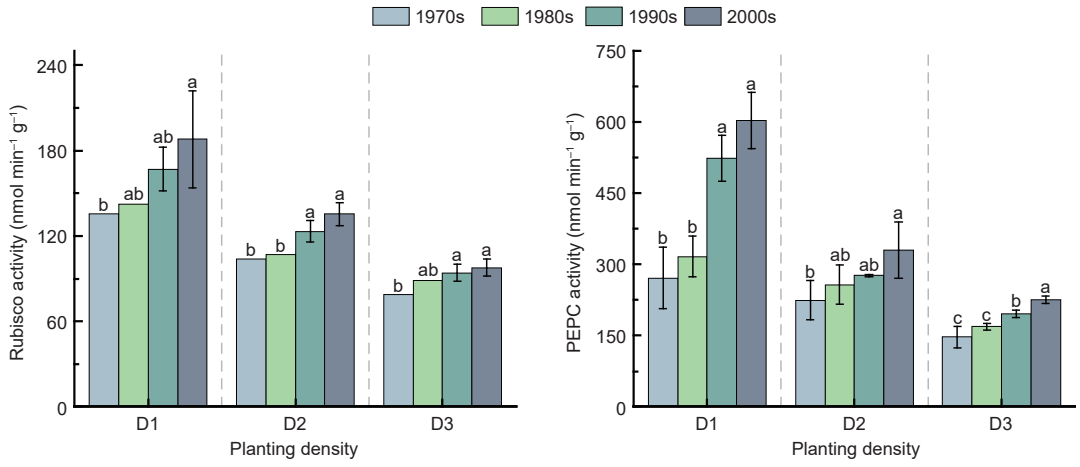


Fig. 3 Trends in photosynthesis key enzymatic activity at silking stage (R1) of maize hybrids of different eras under three planting densities in 2020. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. Bars mean SE (n=3). Values in the same character were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05.

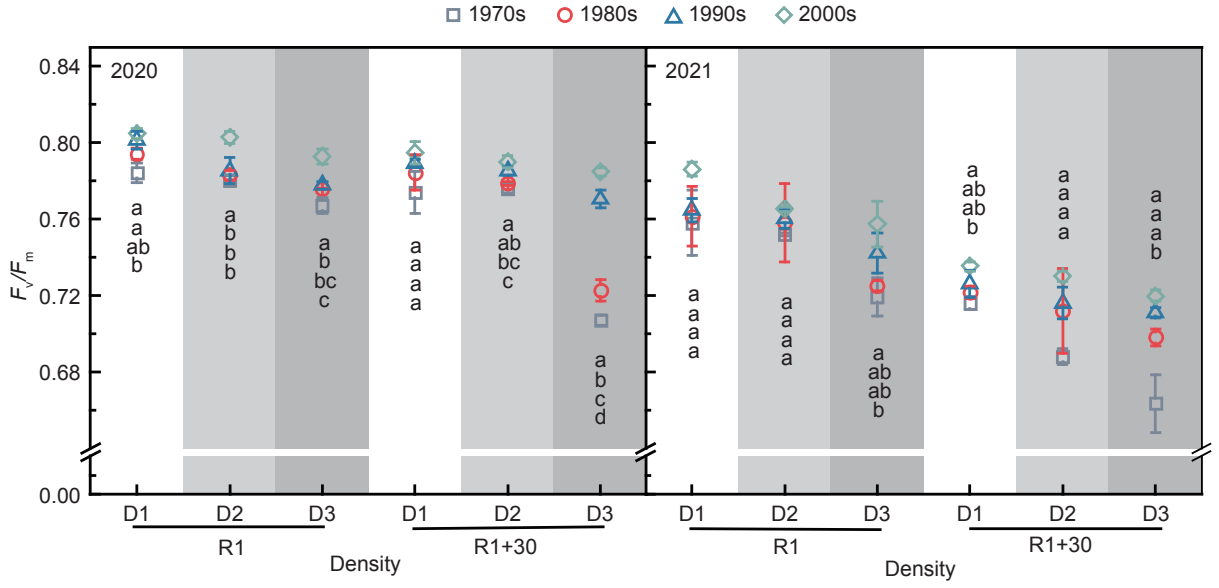


Fig. 4 Changes trend of maximum photochemical efficiency the dark-adapted state of maize hybrids at three planting densities in 2020 and 2021. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. R1, silking stage; R1+30, 20 days after silking. Bars mean SE (n=3). Values in the same character were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05.

prolonged functional longevity, which contributes to enhanced yield potential (Fan *et al.* 2024). The maximum genetic gain in PLA occurred during era E2, following the milky stage; in contrast, no significant increase was observed in the later E3 stage. Similarly, LAD improved significantly during E2, and this period accounted for the majority of total genetic gains in both PLA and LAD. These findings suggest that canopy-related traits were likely a primary focus of varietal improvement efforts during the 1980s. At high density, LA and CLIR showed a decreasing trend across hybrid release eras and were negatively correlated with P_n. In contrast, MULIR exhibited an increasing trend and was significantly positively correlated with grain yield (Appendix G; Fig. 8). A recent study (Ma *et al.* 2022) documented that LA decreased over time from the 1950s to the 2010s, with leaves becoming more erect — particularly the second and third leaves below the ear. Concurrently, the spatial distribution of leaf area increased both above and below the ear in improved cultivars at the same planting density, enhancing light interception through a higher leaf area index (Tollenaar and Lee 2002; Ma *et al.* 2014). These studies collectively provide a robust explanation for the gradual decline in CLIR and concurrent rise in MULIR observed in modern hybrids.

Four groups of genes have been implicated in regulating LA formation and size over decades of breeding (Jafari *et al.* 2024). The greatest genetic gain in LA occurred during era E1, whereas subsequent eras showed comparatively smaller improvements in ear-layer leaf angle (Table 2). Consistent

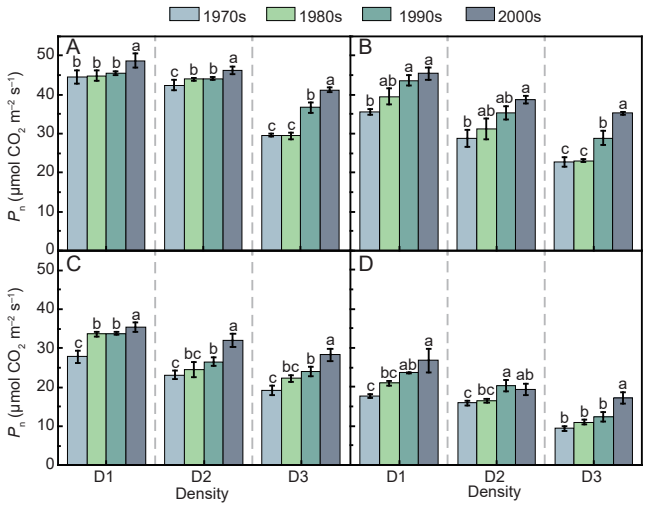


Fig. 5 Changes trend of leaf net photosynthetic rate (P_n) of ear leaf of maize hybrids at three planting densities in 2020 and 2021. A and B are the P_n of ear leaf at silking stage (R1) in 2020 and 2021. C and D are P_n of ear leaf at 20 days after silking (R1+30) in 2020 and 2021, respectively. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. Bars mean SE (n=3). Values in the same character were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05.

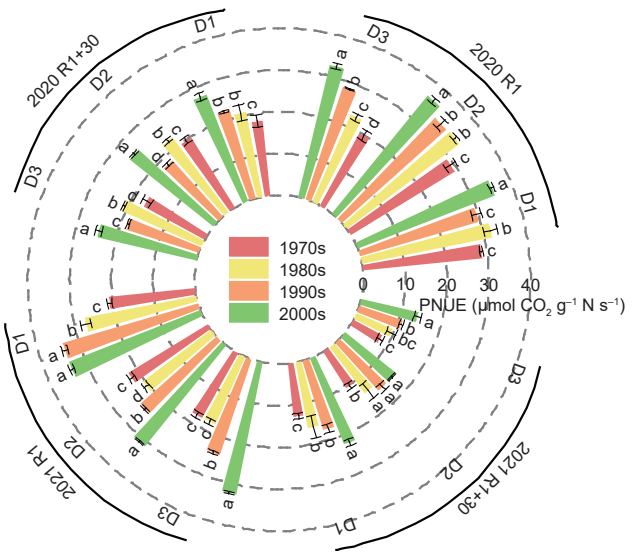


Fig. 6 Changes trend of photosynthetic nitrogen utilization (PNUE) of maize hybrids at three planting densities in 2020 and 2021. R1, silking stage; R1+30, 20 days after silking. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. Bars mean SE (n=3). Values in the same character were marked with different letters to indicate significant differences among hybrids of different eras in the same year and different densities at the level of P<0.05.

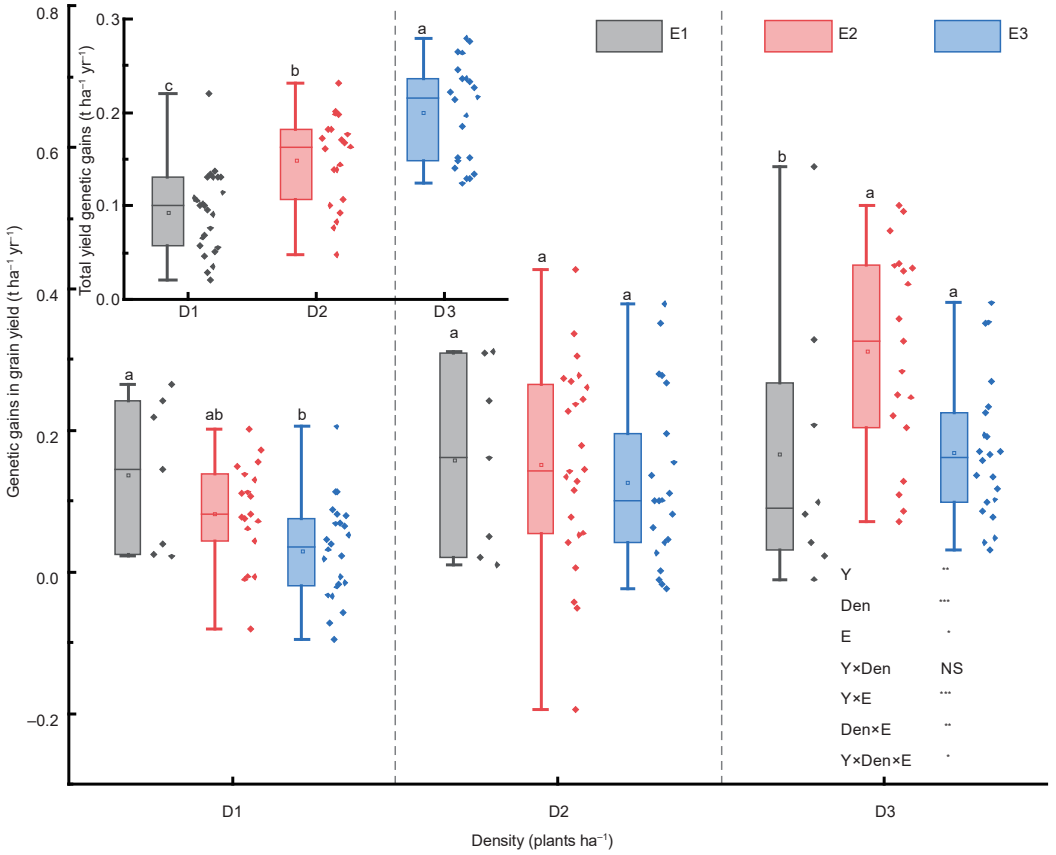


Fig. 7 Comparison of genetic gains in grain yield data for maize hybrids of different ages at three planting densities in 2019, 2020 and 2021. E1–E3, era interval 1, 1970s–1980s; era interval 2, 1980s–1990s; era interval 3, 1990s–2000s. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. Y, year; Den, density; E, era interval. Bars mean SE. NS, not significant (P>0.05); *, **, and ***, significant at P<0.05, P<0.01, and P<0.001, respectively. Values of the same trait were marked with different letters to indicate significant differences among hybrids of different ages in the same year and different densities at the level of P<0.05.

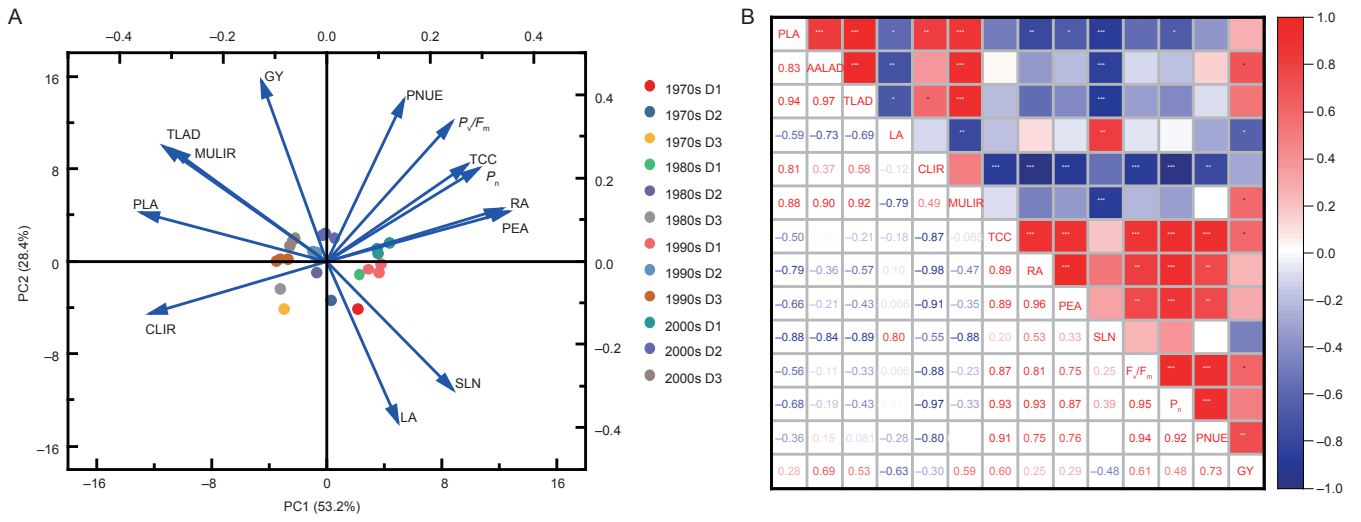


Fig. 8 Principal component analysis (PCA) and correlation analysis for maize yield and photosynthetic indicators. Arrows represent traits and these points represent treatments. PLA, AALAD, TLAD, LA, CLIR, MULIR, TCC, RA, PEA, SLN, P_n, F_v/F_m, PNUE, and GY were population leaf area, leaf area duration after anthesis, total leaf area duration, leaf angle, canopy light interception rate, mid upper layer light interception rate, chlorophyll *a*+*b* content, Rubisco activity, PEPC activity, specific leaf nitrogen, net photosynthetic rate, maximum efficiency of PSII photochemistry, photosynthetic N use efficiency, and grain yield. D1–D3, 45,000, 67,500, and 90,000 plants ha^{-1}, respectively. *, **, and ***, significant at P<0.05, P<0.01, and P<0.001, respectively.

with this, Elli *et al.* (2023) reported that genetic gain in LA between 1980 and 2020 was lower than that achieved from 1930 to 1980, while GY genetic gain remained stable, implying that maize LA may be approaching its physiological limit. Recently, the genetic gain in flag leaves was found to be tenfold greater than that in middle canopy leaves (Elli *et al.* 2023). Modern hybrids possess a smaller LA in the ear layer, which allowed them to achieve a higher gain in the canopy leaves, the canopy leaves are more upright, the CLIR is reduced, and the ear layer is exposed to a greater amount of light radiation (Sangoi e *et al.* 2002; Lacasa *et al.* 2022). Contrastingly, modern hybrids exhibit larger PLA, especially during later reproductive stages, resulting in higher MULIR and consequently maintaining a higher AALAD. Enhanced LAD significantly increased photosynthetic capacity post-silking, particularly during the late grain-filling period (Liu G Z *et al.* 2022). The yield-driven breeding process has selected for maize hybrids with more upright leaves, which confer a more rational plant structure and lower extinction coefficients than old hybrids (Fan *et al.* 2024). Consequently, modern hybrids can establish more vigorous population structures at high densities, delay senescence in lower leaves, and sustain photosynthesis over extended periods favoring the accumulation of dry matter in the population level and ultimately increase in maize population yields (He *et al.* 2022; Jafari *et al.* 2024; Yin *et al.* 2025). Despite sustained genetic gains in maize population traits over the past four decades, we observed relatively limited progress between the 1990s and 2000s compared to earlier periods. In summary, this suggests that improvements in maize population characteristics may have encountered certain physiological or genetic constraints, indicating that future breeding strategies will require deeper physiological insights or more advanced techniques.

4.2. Photosynthetic capacity of ear leaf improved linearly with era of hybrids released

The ear leaf is a critical functional organ in maize yield formation, contributing up to 20% to maize GY (Huang *et al.* 2021). Under dense planting, mutual shading among plants is inevitable, with lower leaves experiencing particularly severe light limitation (Li *et al.* 2014; Guo *et al.* 2021). The resulting reduction in light interception affects their Chl content, P_n and stomatal conductance, thereby limiting photosynthesis (Makoi *et al.* 2010). Furthermore, Chl content and the Chl *a*/*b* ratio decrease significantly with increasing plant density, accompanied by disruption of chloroplast ultrastructure and damage to membrane systems and mitochondria (Griffin *et al.* 2004; Ren *et al.* 2016). In our study, ear leaf chlorophyll content and PKEA tended to increase with the era of hybrid release across all density treatments (Figs. 2 and 3). Concurrently, higher planting densities significantly reduced F_v/F_m, as dense planting impairs the photosynthetic potential of PSII and hinders electron transport, thereby limiting effective solar radiation utilization by ear leaves, which leads to diminished photosynthetic performance, restricted dry matter accumulation, reduced grain weight per plant, and ultimately lower yield per plant (Ren *et al.* 2017b). However, F_v/F_m and P_n in the ear leaves of modern hybrids were significantly higher than those of older hybrids across all densities, and the increase was greater under high-density conditions (Figs. 4 and 5). However, TCC, PKEA, and F_v/F_m increased significantly with hybrid era under high density, indicating that modern hybrids can effectively mitigate the inhibitory effects of density stress on PSII photochemical potential. Enhanced PKEA may compensate for reduced CO_2 assimilation capacity, thereby improving light energy use efficiency and overall photosynthetic performance in summer maize (Ren *et al.* 2017b; Wang *et al.* 2017).

Previous studies indicated that chlorophyll content and PKEA reflect both photosynthetic performance and leaf senescence dynamics, while PEPC and Rubisco are key enzymes responsible for CO_2 fixation, with their activity levels directly influencing photosynthetic rate (Wang *et al*. 2012, 2017). Specifically, in our study, modern hybrids were less adversely affected by planting density than older hybrids and maintained higher photosynthetic performance during mid-reproduction. Likewise, Ding *et al.* (2005) found that older hybrids exhibited irreversible leaf senescence three weeks after flowering, a significant decline in photosynthetic traits. This is due to reduced activities of C_4 enzymes (PEPC and pyruvate orthophosphate dikinase) in senescent leaves as a result of their breakdown (Feller *et al.* 2008; Masclaux-Daubresse *et al.* 2010). In contrast, photosynthetic components in modern hybrids remained functional, sustaining higher P_n (Wang *et al.* 2001), presumably due to higher photosynthetic enzyme activity. Furthermore, leaf photosynthetic capacity indicators were negatively correlated with CLIR (Fig. 8). The main mechanism underlying increased photosynthesis in lower leaves involves enhanced CO_2 diffusion capacity, whereas in upper leaves, it depends on the combined effects of Rubisco catalytic properties and CO_2 diffusion (Yin *et al.* 2019). The lower CLIR and higher MULIR in modern hybrids enable optimal light capture in spike-layer leaves, which also exhibit higher chlorophyll content, PKEA, and F_v/F_m. This integrated advantage enhances photosynthetic performance, increases photosynthetic product formation, promotes dry matter accumulation, and ultimately leads to higher grain yield (Li *et al.* 2019; Cao *et al.* 2021; He *et al.* 2022). Taken together, breeding for dense, high-yielding maize hybrids should prioritize strategies to maintain higher chlorophyll content and PKEA, enhance maximum photochemical efficiency of PSII and P_n under high-density conditions, and extend the duration of these favorable traits in future generations.

4.3. Increase in PNUE of modern hybrids under high density enhanced grain yield

In crop systems, light absorption is largely governed by the targeted regulation of leaf nitrogen (N) content, while improving PNUE requires optimizing the distribution of N within the canopy so that leaf N availability matches intercepted light resources (Moreau *et al.* 2012; Hikosaka 2014). SLN is closely related to nitrogen use efficiency (NUE) in modern hybrids and can be used to estimate grain yield, ear number, and thousand-grain weight at harvest (McCullough *et al.* 1994; Debruin *et al.* 2017). At the same planting density, there was no clear pattern of variation in SLN between the different hybrid generations, although hybrids from the 2000s hybrids were slightly lower than others across all growth stages (Appendix I). However, Chen *et al.* (2017) comparing two groups of U.S. maize hybrids from different eras found a significant reduction in SLN only at a density of 104,000 plants ha^{-1}, and only one older hybrid exhibited significantly lower SLN. Our results indicate that maize hybrids exhibited a progressive increase in PNUE across three densities from the 1970s to the 2000s. Subsequently, modern hybrids demonstrated significantly higher PNUE than older hybrids and maintained elevated PNUE during late reproductive stages under high -density conditions (Fig. 8). Yu *et al.* (2022) showed that optimizing upper-to-middle canopy structure and increasing leaf N content could enhance leaf photosynthetic capacity, thereby substantially improving PNUE in maize. However, earlier studies suggest that to improve PNUE while maintaining or increasing yield, the enhancement in the maximum photosynthetic rate must exceed that of SLN (Yin *et al.* 2019). This underscores the critical trade-off between SLN and P_n. We found that GY increased linearly with the progressive promotion eras of hybrids across all density treatments; however, GY responses to increasing planting density varied among hybrids in different eras (Appendix J). Although AALAD, TCC, and F_v/F_m were all significantly and positively correlated with GY, PNUE exhibited a stronger correlation and emerges as a key indicator for maize hybrid optimization (Fig. 8). The GY of older hybrids (1970s and 1980s) declined with increasing planting density, whereas modern hybrids (1990s and 2000s) were able to maintain or increase yield, reflecting a substantial improvement in density tolerance. Consistent with our findings, similar results were obtained in earlier studies conducted in countries and regions such as Argentina (Di Matteo *et al.* 2016), USA (Chen *et al.* 2017), Brazil (Sangoi *et al.* 2002), Northern Europe (Frei 2000), and North Africa (Badu-Apraku *et al.* 2014).

In this experiment, modern hybrids benefit from two interrelated advantages under high density: first, a more favorable population structure (Fig. 9), which enhances light capture and photosynthetic efficiency in the maize population (Chen *et al.* 2017; Lacasa *et al.* 2022); and second, the ability to maintain sufficient photosynthetic capacity in the ear leaf and sustain high PNUE (Fig. 9), ensuring adequate supply of assimilates to the ears. These traits provide a strong foundation for the accumulation and translocation of photosynthetic products during late reproductive stages, enabling more efficient partitioning of assimilates between source and sink organs and ultimately improve the population yield (Qian *et al.* 2017; Jafari *et al.* 2024). During the grain-filling period, nitrogen is continuously remobilized from leaves to grains for protein synthesis; therefore, maintaining sufficient nitrogen allocation to support ongoing leaf photosynthesis despite declining SLN is crucial (Mu *et al.* 2016). Furthermore, Antonietta *et al.* (2014) found that nitrogen retention in stay-green hybrids at high densities may inhibit N redistribution to grains, potentially limiting grain growth. In our study, SLN in 2000s-era hybrids was lower than in older hybrids across all treatments and stages, while the PNUE was significantly higher than the old hybrids, which may be related to the fact that nitrogen in the leaves of old hybrids could not be effectively utilized and the inability to effectively transfer nitrogen to the grains (Fu *et al.* 2020). Under normal nitrogen supply, PKEA may exceed the threshold required for realizing potential P_n, allowing for reduced nitrogen allocation to chlorophyll and photosynthetic proteins, thereby increasing nitrogen use efficiency (Tazoe *et al.* 2005; Uribelarrea *et al.* 2009; Mu *et al.* 2016). In

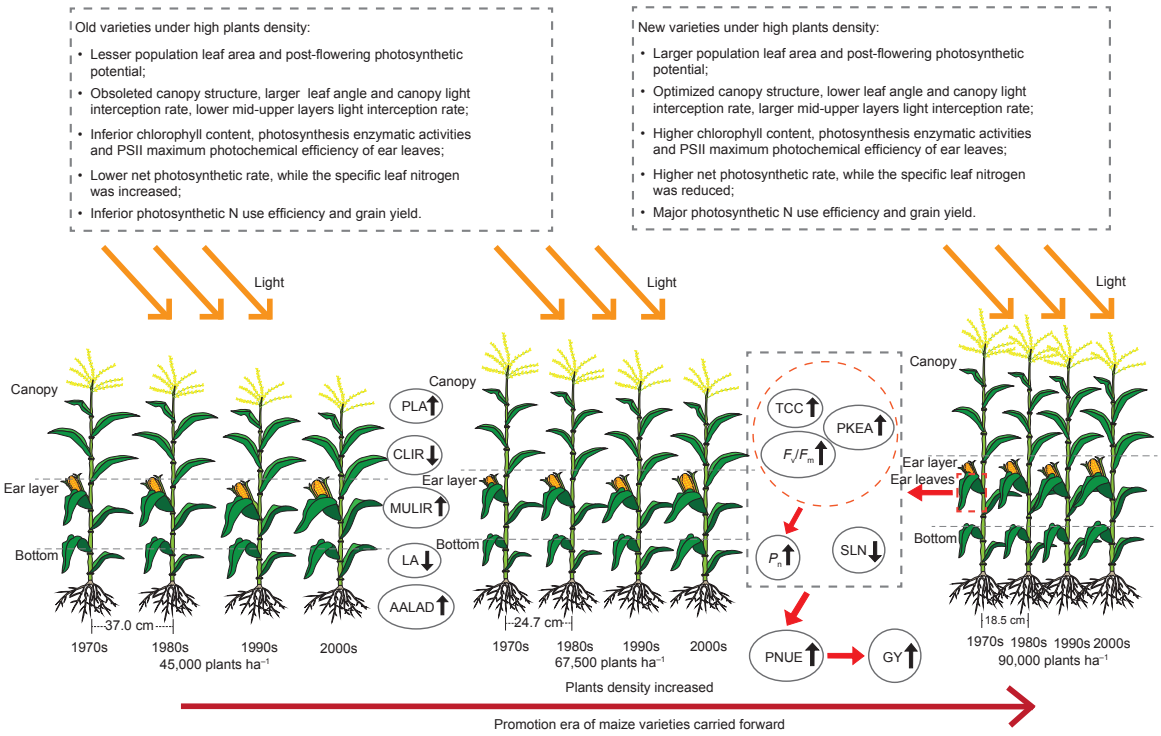


Fig. 9 Scheme to improve population characteristics and photosynthetic capacity under high plant density in maize across four decades. PLA, population leaf area; CLIR, canopy light interception rate; MULIR, mid upper layer light interception rate; LA, leaf angle; AALAD, leaf area duration after anthesis; TCC, chlorophyll *a*+*b* content; PEKA, photosynthesis key enzymatic activity; F_v/F_m, maximum efficiency of PSII photochemistry; P_n, net photosynthetic rate; SLN, specific leaf nitrogen; PNUE, photosynthetic nitrogen utilization efficiency; GY, total leaf area duration, and grain yield.

parallel, the annual genetic gains in PNUE averaged 0.14, 0.60, and 0.62 μmol CO_2 g^{-1} N s^{-1} yr^{-1} over two years across the four eras at D3 density. Importantly, this trend closely mirrored that of yield genetic gain (Fig. 7), clearly indicating that the period from the 1990s to the 2000s saw the most substantial improvement in PNUE. This suggests breeders increasingly recognized the relationship between P_n and yield with leaf N utilization status in maize. Consequently, modern hybrids achieve higher photosynthesis with less leaf nitrogen, and the efficient allocation and utilization of the remaining nitrogen fraction likely contribute to yield gains (Fan *et al*. 2023; Guo *et al*. 2025). Thus, differences in the amount of photosynthetic products and nitrogen transferred to kernels in the nutrient organs of different eras hybrids during late reproductive stages may underlie these improvements, though further research is needed to confirm this mechanism.

5. Conclusion

Maize hybrids in China have steadily improved over the past 40 years. Modern hybrids have fully optimized population characteristics, such as LAD, under high planting densities compared to older hybrids, although the potential for further genetic improvement may now be limited. Concurrently, modern hybrids maintain greater photosynthetic capacity in the ear leaf under high density and during the mid-reproductive stage, as evidenced by higher chlorophyll content, PKEA, F_v/F_m, and P_n. Importantly, modern hybrids can maintain high PNUE under these conditions, thereby possessing higher GY. Therefore, future breeding objectives should continue to focus on optimizing maize population structural characteristics. Simultaneously, emphasis should be placed on enhancing stay-green traits and maintaining leaf nitrogen content to sustain photosynthetic performance under dense planting and ensure sustained PNUE for continued GY improvement.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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