

Post-print: Nitrogen Efficiency Evaluation of Maize Inbred Lines from Shaan A and Shaan B Groups

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Abstract

To elucidate the differential characteristics of nitrogen efficiency among different types of maize inbred lines and screen for nitrogen-efficient maize inbred lines, 33 maize inbred lines selected from Shaan A group and Shaan B group were used as materials, with four backbone inbred lines (‘Zheng 58’ , ‘Chang 7-2’ , ‘PH6WC’ , and ‘PH4CV’) as controls. Physiological indicators including ear leaf SPAD value, leaf area, dry matter accumulation, and nitrogen content in leaves, stems, and grains of maize inbred lines were investigated under two fertilization conditions [0 kg(N) · hm², 180 kg(N) · hm²]. Principal component analysis and fuzzy membership function were employed, and stepwise regression analysis was used to establish an optimal regression equation for screening comprehensive evaluation indicators of low-nitrogen tolerance. The results showed that ear leaf SPAD value, green leaf area at silking stage, stem dry weight at silking stage, leaf dry weight at silking stage, and grain nitrogen content could serve as secondary trait screening indicators for low-nitrogen tolerance in maize inbred lines. Using yield as the primary trait indicator, the 37 maize inbred lines could be classified into 14 high-yield nitrogen-efficient types, 5 low-yield nitrogen-efficient types, 15 low-yield nitrogen-inefficient types, and 3 high-yield nitrogen-inefficient types. Based on screening with the comprehensive low-nitrogen tolerance value D, the 37 maize inbred lines could be divided into three categories: 15 with strong low-nitrogen tolerance (D value ≥ 0.5), 15 with moderate low-nitrogen tolerance (0.35 ≤ D value < 0.5), and 7 with poor low-nitrogen tolerance (D value < 0.35). Comprehensive analysis revealed that under both nitrogen application conditions, five maize inbred lines ‘KB215’ , ‘KB417’ , ‘KA225’ , ‘KB081’ , and ‘L123098-2’ exhibited characteristics of large green leaf area at silking stage, high stem and leaf dry weight at silking stage, high grain nitrogen content, high grain yield, and strong low-nitrogen tolerance.

Therefore, strengthening selection pressure in breeding environments and implementing low-nitrogen selection strategies can effectively improve nitrogen use efficiency in maize germplasm.

Full Text

Evaluation of Nitrogen Efficiency for Selected Shaan A and B Maize Inbred Lines

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Abstract

To elucidate the differential characteristics of nitrogen efficiency among different types of maize inbred lines and screen for high nitrogen-efficient germplasm, we evaluated 33 maize inbred lines selected from Shaan A and B groups, using four elite inbred lines (‘Zheng 58’ , ‘Chang 7-2’ , ‘PH6WC’ , and ‘PH4CV’) as controls. Physiological indicators including SPAD values of ear leaves, leaf area, dry matter accumulation, and nitrogen content in leaves, stems, and grains were measured under two nitrogen fertilization regimes [0 kg(N) · hm² and 180 kg(N) · hm²]. Using principal component analysis and fuzzy membership functions, we established an optimal regression equation through stepwise regression analysis to identify comprehensive evaluation indices for low-nitrogen tolerance. The results indicated that SPAD values of ear leaves, green leaf area at silking, stem dry weight at silking, leaf dry weight at silking, and grain nitrogen content could serve as secondary screening indicators for low-nitrogen tolerance in maize inbred lines. Using yield as the primary indicator, the 37 inbred lines were classified into four types: 14 as high-yield and high-nitrogen-efficiency, 5 as low-yield and high-nitrogen-efficiency, 15 as low-yield and low-nitrogen-efficiency, and 3 as high-yield and low-nitrogen-efficiency. Based on the comprehensive low-nitrogen tolerance value (D-value), the 37 lines were divided into three categories: 15 lines with strong low-nitrogen tolerance (D-value > 0.5), 15 lines with moderate tolerance (0.35 < D-value < 0.5), and 7 lines with poor tolerance (D-value < 0.35). Comprehensive analysis under both nitrogen conditions revealed that five inbred lines— ‘KB215’ , ‘KB417’ , ‘KA225’ , ‘KB081’ , and ‘L123098-2’ —exhibited large green leaf area at silking, high stem and leaf dry weight, high grain nitrogen content, high grain yield, and strong low-nitrogen tolerance. These findings demonstrate that strengthening selection pressure under breeding environments and implementing low-nitrogen selection strategies can effectively improve nitrogen utilization efficiency in maize germplasm.

Keywords: Maize; Inbred line; Nitrogen efficiency; Principal component anal-

ysis; Membership function

Introduction

Nitrogen, as an essential macronutrient for plant growth, plays a critical role in plant development. China has become the world's largest producer and consumer of nitrogen fertilizer, accounting for one-third of global consumption in 2012, yet nitrogen use efficiency (NUE) remains only 30–40%, far below the world average of 50%. Excessive nitrogen application increases production costs, exacerbates environmental pollution, and reduces nitrogen utilization efficiency in maize (*Zea mays*). Consequently, reducing nitrogen fertilizer input while improving NUE has become a research priority. Previous studies have demonstrated significant genotypic variation in nitrogen use efficiency among different maize varieties. Duvick suggested that increased maize yields in the United States primarily resulted from enhanced resistance to environmental stresses, with yield essentially representing stress tolerance. Improvement in maize yield has been attributed to better stress tolerance, and the stress resistance of hybrids originates from their parental inbred lines. Therefore, evaluating genotypic differences in nitrogen efficiency among maize germplasm, identifying nitrogen-efficient genetic resources, and subsequently breeding nitrogen-efficient varieties represent effective approaches for improving nitrogen utilization efficiency.

Researchers have employed various evaluation indicators to classify different nitrogen efficiency types. Liu et al. categorized varieties into four types based on nitrogen efficiency and nitrogen response: high efficiency under both high and low nitrogen, high efficiency under low nitrogen only, high efficiency under high nitrogen only, and low efficiency under both conditions. Zhang et al. analyzed low-nitrogen tolerance in 30 maize varieties using thousand-kernel weight, ear length, and kernel number per ear as indicators, identifying 'Zhengdan 958', 'Shandan 8815', and 'Jingke 28' as relatively low-nitrogen-tolerant varieties. Cui et al. classified inbred lines into four types based on yield under high nitrogen and low-nitrogen stress index: high efficiency under both conditions, high yield with low nitrogen efficiency, low yield with high nitrogen efficiency, and low yield with low nitrogen efficiency. Wang et al. used grain weight and nitrogen use efficiency for grain production as classification criteria.

Our research group constructed two heterotic groups—Shaan A group and Shaan B group—with the primary objective of enhancing adaptability. Through a technical approach involving multi-location trials, high planting density, reduced fertilization, and limited irrigation, we developed the KA and KB series of maize inbred lines. This study utilized 33 selected maize inbred lines from these groups, with four elite inbred lines as controls, to evaluate nitrogen efficiency across different genotypes by analyzing various morphological and physiological indicators. The objectives were to elucidate the physiological basis of nitrogen efficiency differences among different maize inbred line types, identify reliable screening indicators, and select nitrogen-efficient inbred lines to provide a scientific basis for further optimization of the Shaan A and B heterotic groups and

development of nitrogen-efficient maize breeding programs.

1.1 Experimental Materials

Thirty-three maize inbred lines selected from Shaan A and B groups served as experimental materials, with four elite inbred lines (‘Zheng 58’ , ‘Chang 7-2’ , ‘PH6WC’ , and ‘PH4CV’) as controls. The 33 inbred lines originated from two heterotic populations (Shaan A and B groups) constructed by Northwest A&F University. The breeding strategy simplified the heterotic pattern while intensifying artificial selection under stress conditions through a technical route of multi-location trials, high density, reduced fertilization, and limited irrigation. From 2009 to 2013, five rounds of joint improvement were conducted across 30 locations. Selection was based on single-ear grain weight (>100 g), kernel extraction rate ($>87\%$), and number of selected ears per row (>3 ears) through continuous selfing in Shaanxi and Hainan (two generations per year). Detailed information on the materials is provided in Table 1 .

1.2 Experimental Design

The experiment was conducted in 2014 and 2015 at the Yangling Maize Experimental Station of Northwest A&F University ($34^{\circ}54$ N, $108^{\circ}7$ E). The experimental field had loam soil that had been subjected to low-nitrogen stress since 2003, with uniform fertility. The 0–20 cm soil layer contained organic matter $10.2 \text{ g} \cdot \text{kg}^{-1}$, total nitrogen $1.06 \text{ g} \cdot \text{kg}^{-1}$, available nitrogen $35.4 \text{ mg} \cdot \text{kg}^{-1}$, available phosphorus $14.27 \text{ mg} \cdot \text{kg}^{-1}$, and available potassium $151.4 \text{ mg} \cdot \text{kg}^{-1}$.

Two nitrogen application levels were established: $0 \text{ kg} \cdot \text{hm}^{-2}$ (low nitrogen, N0) and $180 \text{ kg} \cdot \text{hm}^{-2}$ (normal nitrogen, N180). A split-plot design was employed with nitrogen treatment as the main plot and inbred line as the subplot, with three replications. Each inbred line was planted in two rows, 5 m long with 60 cm row spacing, resulting in a plot area of 6 m^2 and planting density of $60,000 \text{ plants} \cdot \text{hm}^{-2}$. Forty percent of nitrogen fertilizer was applied as basal fertilizer, with the remaining 60% top-dressed at the 7-leaf stage. Other management practices followed standard field procedures.

1.3 Measurement Items and Methods

At the jointing stage (7-leaf stage), 10 uniform plants were tagged for leaf age and plant measurements, with subsequent investigations following the growth progression. SPAD values of ear leaves were measured between 9:00–10:00 AM on clear days at silking stage using a SPDA-502plus chlorophyll meter, with 10 plants measured per line and averages calculated. Green leaf area was measured in five plants at silking stage using the formula: leaf area = leaf length \times leaf width $\times 0.75$.

At silking and maturity stages, three representative plants per inbred line were selected to determine dry matter accumulation. Plants were separated into stems, leaves, husks, cobs, and kernels, killed at 105°C for 30 minutes, then

oven-dried at 80°C to constant weight for dry weight calculation. Dried samples were ground, and 0.5 g was used to determine nitrogen content in each organ using a Kjeltac 8200 automatic Kjeldahl nitrogen analyzer.

At harvest, all ears in each plot were collected for yield measurement, calculated at 14% moisture content.

1.4 Data Processing

The low-nitrogen tolerance index was calculated as:

$$\text{Low-nitrogen tolerance index} = \left(\frac{\text{Value under low nitrogen}}{\text{Value under normal nitrogen}} \right) \times 100\% \quad (1)$$

Following the method of Hu et al., membership functions, principal component weights, and comprehensive low-nitrogen tolerance values were analyzed. The membership function value for each inbred line was calculated as:

$$\mu(x_i) = \frac{x_i - x_{i\min}}{x_{i\max} - x_{i\min}} \quad (i = 1, 2, 3, \dots, n) \quad (2)$$

where $\mu(x_i)$ is the membership function value for the i th principal component, x_i is the score of each material for a given principal component, and $x_{i\max}$ and $x_{i\min}$ are the maximum and minimum values of the i th principal component across all inbred lines.

The weight of each principal component (W_i) was calculated as:

$$W_i = \frac{P_i}{\sum_{i=1}^n P_i} \quad (i = 1, 2, 3, \dots, n) \quad (3)$$

where W_i represents the importance of the i th principal component among all components, and P_i is the contribution rate of the i th principal component.

The comprehensive low-nitrogen tolerance value (D) for each inbred line was calculated as:

$$D = \sum_{i=1}^n [\mu(x_i) \cdot W_i] \quad (i = 1, 2, 3, \dots, n) \quad (4)$$

Microsoft Excel 2007 was used for data organization and calculation of trait means. Origin 9.0 software was used for figure preparation. SPSS 20.0 software was used for statistical analysis, including correlation coefficients, principal component analysis, and stepwise regression analysis.

2.1 Effects of Different Nitrogen Levels on Maize Inbred Line Yield

As shown in Figure 1 [Figure 1: see original paper], significant yield variations existed among maize inbred lines under different nitrogen levels. Using the

average yield of all inbred lines under both nitrogen conditions as coordinate axes, the materials could be classified into four types. Fourteen inbred lines, including 'KB081', 'KA225', 'KB215', and 'KB417', fell in the first quadrant with yields above the mean under both nitrogen levels, representing the high-yield and high-nitrogen-efficiency type. Five lines, including 'L123094-4' and '2014KB-54', fell in the second quadrant with yields above the mean only under low nitrogen, representing the low-yield and high-nitrogen-efficiency type. Fifteen lines, including 'KB080', '2011KB-14', and '2014KA-60', fell in the third quadrant with yields below the mean under both conditions, representing the low-yield and low-nitrogen-efficiency type. Three lines, including 'KB106', 'KB204', and '2013YLD3A1-5', fell in the fourth quadrant with yields above the mean only under normal nitrogen, representing the high-yield and low-nitrogen-efficiency type.

2.2 Effects of Different Nitrogen Levels on Physiological Indicators of Maize Inbred Lines

Analysis of variance showed that physiological indicators including SPAD values, leaf area, dry matter accumulation, and nitrogen accumulation reached significant or highly significant levels between treatments and among inbred lines (Table 2). Under different nitrogen levels, the coefficient of variation for all traits except green leaf area exceeded 15%, indicating substantial variation among inbred lines and facilitating evaluation and selection.

Using trait values under normal nitrogen as the x-coordinate and those under low nitrogen as the y-coordinate, with their means as central lines (Figure 2 [Figure 2: see original paper]), inbred lines could be classified into four types based on different trait means. Comprehensive analysis revealed that five inbred lines—'2013KB-47', '2013XCA1-1', 'KB081', '2014YLKA-58', and 'PH6WC'—frequently appeared in the first quadrant across multiple indicators including ear leaf SPAD value, green leaf area at silking, stem dry weight at silking, leaf dry weight at silking, post-silking dry matter accumulation, and grain nitrogen content, indicating values above the mean under both nitrogen conditions (high nitrogen efficiency type). In contrast, five lines—'KB043', 'KB204', '2013KA-34', 'KB106', and 'PH4CV'—frequently appeared in the third quadrant, showing values below the mean under both conditions (low nitrogen efficiency type).

2.3 Principal Component Analysis of Nitrogen Efficiency-Related Traits in Maize Inbred Lines

Since relative values of various traits showed varying degrees of correlation, potentially affecting low-nitrogen tolerance evaluation, trait relative values (low-nitrogen tolerance indices) were used to comprehensively evaluate low-nitrogen tolerance while eliminating genotypic differences. Correlation analysis of 10 trait relative values (Table 3) showed six pairs of traits were highly significantly correlated ($P < 0.01$) and five pairs were significantly correlated ($P < 0.05$).

Except for the significant negative correlation between total nitrogen content at silking and post-silking nitrogen accumulation (-0.366), significant positive correlations were observed between stem dry weight at silking and leaf dry weight at silking, leaf nitrogen content at silking and grain nitrogen content, leaf dry weight at silking and total dry weight at silking, leaf nitrogen content at silking and grain nitrogen content, post-silking dry matter accumulation and post-silking nitrogen accumulation and grain nitrogen content, leaf nitrogen content at silking and total nitrogen content at silking, and post-silking nitrogen accumulation and grain nitrogen content, with correlation coefficients ranging from 0.331 to 0.512. These results indicate overlapping information among traits due to significant correlations.

To eliminate this effect, principal component analysis was performed on relative values of 10 traits to comprehensively evaluate low-nitrogen tolerance among different inbred lines. Table 4 shows that the first four principal components accounted for 72.288% of cumulative variance, representing 72.288% of the total information. The principal component matrix reflects the relative loading magnitude and direction of each trait on the components, indicating their influence. Based on eigenvector loadings of the first four principal components: The first component contributed 22.780% of variance, with post-silking dry matter accumulation, post-silking nitrogen accumulation, and grain nitrogen content showing greater eigenvalues than other traits.

2.4 Nitrogen Efficiency Analysis of Different Maize Inbred Lines

Using the contribution rates of the four principal components, weight coefficients were calculated as (0.315, 0.292, 0.219, 0.174). Combined with factor scores, the comprehensive low-nitrogen tolerance value (D) for each inbred line was calculated. Higher D-values indicate stronger low-nitrogen tolerance. Based on D-values, inbred lines were classified into three grades: Grade I ($D \geq 0.5$) as strong low-nitrogen tolerance, Grade II ($0.35 \leq D < 0.5$) as moderate tolerance, and Grade III ($D < 0.35$) as poor tolerance. Accordingly, 37 inbred lines were divided into three types, with 15 lines including 'KB417', 'L123098-2', '2013HXB1-4', and 'KB106' having D-values > 0.5 , representing strong low-nitrogen tolerance (Table 5), while seven lines including '2014KA-60', '2013KA-34', and 'KB043' showed poor tolerance.

An optimal regression equation was constructed using the comprehensive D-value and phenotypic trait relative values to screen for low-nitrogen tolerance indicators. With D-value as the dependent variable (y) and relative values of 10 phenotypic traits as independent variables, stepwise regression analysis yielded:

$$y = -0.966 + 0.358X_1 + 0.569X_2 + 0.212X_3 + 0.197X_4 + 0.160X_{10} \quad (5)$$

where X_1 , X_2 , X_3 , X_4 , and X_{10} represent relative values of SPAD value, green leaf area at silking, stem dry weight at silking, leaf dry weight at silking, and

grain nitrogen content, respectively. The equation's coefficient of determination (R^2) was 0.977, indicating these five traits explained 97.7% of total variation. The F-value was 132.89 ($P = 0.0001$), confirming equation significance. Therefore, relative values of SPAD value, green leaf area at silking, stem dry weight at silking, leaf dry weight at silking, and grain nitrogen content significantly affected the comprehensive D-value and could serve as secondary screening indicators for low-nitrogen tolerance in maize inbred lines.

Using yield as the primary indicator, 12 high-yield and high-nitrogen-efficiency inbred lines were identified (Figure 1). Combined with 12 lines showing strong low-nitrogen tolerance based on D-values (Table 5), five inbred lines— 'KB215' , 'KB417' , 'KA225' , 'KB081' , and 'L123098-2' —demonstrated high yield under both nitrogen conditions, high SPAD values at silking, large green leaf area, high stem and leaf dry weight, high grain nitrogen content, and strong low-nitrogen tolerance.

3 Discussion and Conclusion

Zhang et al. used low-nitrogen stress indices as relative values of maize phenotypic traits to identify screening indicators for low-nitrogen tolerance. Feng et al. used relative values of traits showing maximum variation under nitrogen stress for variety screening through membership function values. Chen and Chen classified nitrogen efficiency types in maize inbred lines based on yield under high and low nitrogen, reporting that elite lines 'PH6WC' and 'Chang 7-2' were high-efficiency under both conditions, 'Zheng 58' was high-efficiency under low nitrogen only, and 'PH4CV' was low-efficiency under both conditions. Cui used yield under high nitrogen and low-nitrogen stress indices, classifying 'Zheng 58' and 'PH6WC' as high-efficiency types and 'Chang 7-2' and 'PH4CV' as low-yield but high-efficiency types. Our study, based on 33 inbred lines from Shaan A and B groups developed through multi-location, high-density, low-nitrogen, and drought conditions, identified 15 lines with strong low-nitrogen tolerance using the comprehensive D-value as a secondary indicator, which largely agreed with yield-based classification. Among the control elite lines, 'Chang 7-2' and 'PH6WC' were high-yield and high-efficiency types, 'Zheng 58' was low-yield but high-efficiency, and 'PH4CV' was low-yield and low-efficiency, consistent with Chen and Chen's results and validating the D-value approach for evaluating low-nitrogen tolerance.

Establishing and evaluating low-nitrogen tolerance indicator systems remains a research focus. Liu et al. proposed using yield traits as screening indicators, but nitrogen affects multiple plant aspects, and relying solely on yield may introduce bias from environmental and climatic factors, while considering all related traits increases screening difficulty. Lafitte and Edmeades suggested that under both low and high nitrogen, ear leaf chlorophyll concentration, leaf area, and plant height could serve as selection indicators for improving nitrogen efficiency. Cui et al. demonstrated that grain nitrogen uptake and total nitrogen in stems and leaves at silking were important indicators for screening nitrogen-efficient

genotypes. Our study analyzed traits showing significant differences between nitrogen treatments and among inbred lines (facilitating inter-line comparison), calculated the comprehensive D-value, and performed regression analysis between D-values and relative trait values. The results identified SPAD value, green leaf area at silking, stem dry weight at silking, leaf dry weight at silking, and grain nitrogen content as major factors influencing D-value, suitable as secondary screening indicators for low-nitrogen tolerance.

Under optimal conditions, inter-plant variation is minimal and differences are not obvious. However, under stress conditions, individual differences are amplified, increasing selection efficiency. Constructing maize populations with high efficiency for ecological factors (light, temperature, water, CO₂) and resource supply requires weak competition, good stress adaptability, and efficient resource utilization under scarce conditions while minimizing inter-plant competition. Modern maize improvement should modify breeding selection methods, strengthen stress selection, and implement low-nitrogen selection strategies to enhance stress adaptability. Our two-nitrogen-level experiment clarified nitrogen efficiency differences among 33 inbred lines, identified nitrogen-efficient germplasm, and confirmed the feasibility of selecting nitrogen-efficient maize through low-nitrogen stress. However, this study only analyzed grain yield and related physiological indicators, while nitrogen efficiency in maize inbred lines is influenced by genetics, breeding, physiology, and ecology. Multi-year, multi-location systematic studies from different perspectives, levels, and scales are needed for comprehensive evaluation and to further elucidate the importance of stress selection.

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