

Comprehensive Evaluation of Biological Fresh Weight Yield-related Characteristics of Silage Maize (*Zea mays*) at Maturity Stage

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Biological yield and quality are critical indicators for evaluating silage corn (*Zea mays*). Among these, biological yield is closely associated with multiple traits of the crop. This study recorded data of 10 traits over two years for 37 silage corn varieties cultivated in hilly mountainous regions of China. Multivariate analysis revealed correlations among all 10 traits. Using correlation data, principal component analysis, cluster analysis, and ridge regression were applied to classify the 37 silage corn varieties into six distinct groups. Key findings identified plant height, ear height, greenness retention rate, and dry weight as critical variables for developing a mathematical model to evaluate silage corn yield and estimate its biological fresh weight. Results indicated that when screening for high-biological-fresh-weight silage corn varieties, priority should be given to those with longer growing periods, compact plant types, superior greenness retention, and higher dry weight. Finally, comparative analysis of biological yields of high-yielding silage corn in Sichuan Province, China, provided actionable references for optimizing silage corn cultivation in local hilly regions.

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Keywords: Maize silage; Biological yield; Biological character; Comprehensive evaluation; Principal component analysis

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INTRODUCTION

Silage maize (*Zea mays*) usually refers to whole maize plants, including maize ears, that are harvested during the kernel milk and dough stages using specialized harvesters and are then used to produce feed for herbivorous livestock through chopping and fermentation (Ghany *et al.* 2020; Bakri 2021; Al-Rajhi *et al.* 2023). In China, however, silage maize is categorized into silage-specific, dual-purpose (with mature ears for grain and stover for silage), and general-purpose (edible for both humans and livestock) (Pan *et al.* 2002). Silage maize exhibits high biological yield, favorable fiber quality, and superior greenness retention (Contreras-Covea *et al.* 2009); among these, the biological fresh weight (FW) yield is an important evaluation factor.

The southwestern region of China has abundant rainfall, and the corn plants grow well in the early stage. However, due to the short duration of sunlight and the high temperature and humidity in summer in this area, more diseases and pests occur, resulting in lower yields compared to those in the north. To cultivate more high-yield silage maize

varieties, breeders usually consider traits that affect the biological yield of maize as the evaluation object in the selection of inbred lines (Barrière *et al.* 1997). Correlation analysis between agronomic characteristics and the biological yield of silage maize showed that plant height, harvest date, ear height, 1,000-grain weight, and ear length were the main factors affecting the biological yield of silage maize (Fromme *et al.* 2019).

This study has previously focused on investigating yield-related traits in both silage corn (*Zea mays*) and grain corn, examining correlations between single-plant yield and key traits such as 1000-grain weight, kernel rows per ear, and growing period in mountainous regions (Long *et al.* 2024). These studies identified critical yield-determining factors, providing a theoretical foundation for local corn breeding programs. Building on this foundational work, previous investigations have highlighted the substantial influence of the corn ear on silage corn biomass yield (Coors *et al.* 1997). Concurrently, nutrient accumulation and remobilization in maize are regulated by growth stages and maturity levels, ultimately emerging as primary determinants of its biological yield (Kim *et al.* 2001). Extending prior findings, the present study conducts a more detailed analysis of two specific traits—setting percentage (SP) and double ear rate (DE), while also evaluating correlations and yield contributions of 10 additional traits, including plant type (PT), greenness retention (GR) characteristics.

EXPERIMENTAL

Test Material

The 37 maize silage varieties were provided by 17 institutions and companies, as detailed in Table 1.

Test Design

The 37 varieties were cultivated in Nanchong (Sichuan province, China; 30.6°N, 105.3°E; elevation 361 m) for a 2-year trial (2019 and 2020). Each year adopted a randomized block design approach with three replicates. Each block had an area of 20 m² and contained five rows, with a density of 60,000 plants per hectare. The block yield was based on the harvest of the middle three rows. In addition, at least four protection rows of maize were planted around the experimental sites.

Test Method

The growth period (GP) was recorded after sowing, and physiological maturity was considered when 60% of the ears of the same material formed a black layer (the period when small black spots appear on the roots of corn kernels). Harvesting was conducted at physiological maturity (Khan *et al.* 2012). At harvest, setting percentage (SP), double ear rate (DE), stalk-lodging rate (SL), plant type (PT), greenness retention (GR), plant height (PH), and ear height (EH) were noted. Additionally, biological FW based on 10 continuously selected whole plants and biological dry weight (DW) were determined. And biological dry weight (DW) was measured after drying at 105°C for 15 min and 80°C to constant weight (DHG-9240A; Shanghai Kailang Instrument Equipment Factory, Shanghai, China) (Dong *et al.* 2006).

Statistical Analysis

The data on plant types and GR are descriptive and were quantified as categorical data. Through quantification, all trait data were standardized (plant type: 1 = loose, 2 = flat, 3 = semi-compact, 4 = compact; greenness retention: 1 = poor, 2 = average, 3 = green, 4 = excellent).

The average values of the 37 maize silage varieties over two years were calculated using Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA, USA). Data standardization, principal component analysis (Patto *et al.* 2009), and verification analysis were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Ridge regression analysis was performed using SPSSPRO (Suzhou Zhongyan Network Technology Co., Ltd., Guangzhou, China). Cluster and correlation analyses were conducted, and box plots were constructed using Origin 2022b (OriginLab, Northampton, MA, USA).

Table 1. 37 Maize Silage Varieties and Sources

Serial number	Varieties	Sources
1	Nanqing 385	Nanchong Academy of Agricultural Sciences
2	Nanqing 232	Nanchong Academy of Agricultural Sciences
3	Nanqing 2142	Nanchong Academy of Agricultural Sciences
4	Nanqing 2088	Nanchong Academy of Agricultural Sciences
5	Nan W816	Nanchong Academy of Agricultural Sciences
6	Nan W3465	Nanchong Academy of Agricultural Sciences
7	Nanqing 77	Nanchong Academy of Agricultural Sciences
8	Nanqing 521	Nanchong Academy of Agricultural Sciences
9	Longping 559	Anhui Longping Hi-Tech Seed Co., Ltd.
10	Guangqing 203	Guangyuan Academy of Agricultural Sciences
11	Guangqing 1802	Guangyuan Academy of Agricultural Sciences
12	Le 1999	Leshan Academy of Agricultural Sciences
13	Liang 2020	Xichang Academy of Agricultural Sciences of Liangshan Prefecture
14	Mian 2005	Mianyang Academy of Agricultural Sciences
15	Miandan 968	Mianyang Academy of Agricultural Sciences
16	Miandan 72	Mianyang Academy of Agricultural Sciences
17	Mian 1902	Mianyang Academy of Agricultural Sciences
18	Jishengyu 885	Santai Dasheng Maize Research Institute
19	Jishengyu 85	Santai Dasheng Maize Research Institute
20	Derui 986	Sichuan Deruifudun Agricultural Science and Technology Co., Ltd.
21	Jinliugu 20	Sichuan Jinliugu Seed Co., Ltd.
22	Chuandan 920	Maize Research Institute of Sichuan Agricultural University
23	Chuandan 2110	Maize Research Institute of Sichuan Agricultural University
24	Chuandan 919	Maize Research Institute of Sichuan Agricultural University
25	Zhongya 253	Sichuan Jialing Crop Variety Research Co., Ltd.
26	Shengkeqingzhu 2020	Institute of Biological and Nuclear Technologies of Sichuan Academy of Agricultural Sciences
27	Chengdan 719	Crop Research Institute of Sichuan Academy of Agricultural Sciences
28	Chengdan 608	Crop Research Institute of Sichuan Academy of Agricultural Sciences
29	Chengdan 3601	Crop Research Institute of Sichuan Academy of Agricultural Sciences
30	Chengdan 768	Crop Research Institute of Sichuan Academy of Agricultural Sciences

31	Chengdan 623	Crop Research Institute of Sichuan Academy of Agricultural Sciences
32	Chengdan 385	Crop Research Institute of Sichuan Academy of Agricultural Sciences
33	Chengdan 3211	Crop Research Institute of Sichuan Academy of Agricultural Sciences
34	Chengqing 366	Crop Research Institute of Sichuan Academy of Agricultural Sciences
35	Haiyu 1	Sichuan Shudi Seed Co., Ltd.
36	Yayuqingzhu 8	Sichuan Yayu Science and Technology Development Co., Ltd.
37	Xikangyu 191	Ya'an Academy of Agricultural Sciences

Comprehensive Evaluation of Silage Maize Value

The comprehensive analysis was performed using the following equations,

$$U(X_j) = (X_j - X_{min}) / (X_{max} - X_{min}), j=1,2,\dots,n, \quad (1)$$

$$W_j = P_j / \sum_{j=1}^n P_j, \quad j = 1, 2, \dots n \quad (2)$$

where X_j represents the j^{th} comprehensive trait, $U(X_j)$ represents the membership function value of the j^{th} comprehensive trait, X_{max} and X_{min} represent the minimum and maximum values of the j^{th} comprehensive trait, respectively (Xue *et al.* 2013), W_j represents the importance degree (weight) of the j^{th} comprehensive trait among all comprehensive traits, and P_j represents the contribution rate of the j^{th} comprehensive trait of maize silage obtained through principal component analysis.

The comprehensive evaluation value (D) for each maize was calculated by Eq. 3.

$$D = \sum_{j=1}^n U(X_j) \times W_j, \quad j = 1, 2, \dots n \quad (3)$$

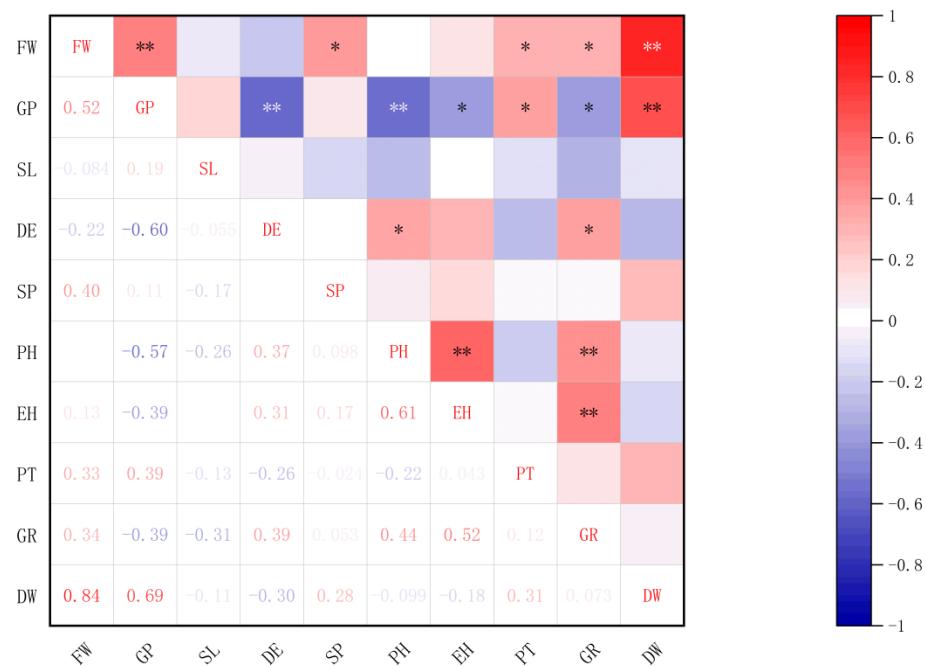
RESULTS AND DISCUSSION

Correlation Analysis

Table 2 shows the basic statistics for the 10 characteristics of 37 maize silage varieties, including the minimum and maximum values, arithmetic mean, and standard deviation. Correlations with different strengths were observed among the 10 characteristics (Fig. 1). Colour depth indicates the significance of the degree of correlation between two characteristics; the darker the colour, the more significant it is. The correlations of various maize silage traits were different and complicated. Among them, positive correlations were high between biological FW and biological DW, biological DW and GP, biological FW and GP, PH and EH, PH and GR, and EH and GR. Negative correlations were observed among GP, PH, GP, and the DE values. Therefore, principal component and cluster analyses should be performed using the correlation of single traits to analyse biological yield.

Table 2. Basic Statistics for 10 Characteristics of Maize Silage Varieties

Variables	Minimum	Maximum	Mean	SD
biological fresh weight (FW, kg/hm ²)	42493.82	60057.62	51134.11	284.77
growth period (GP, day)	105.20	120.78	113.05	5.22
stalk-lodging rate % (SL)	0.00	12.23	3.24	2.41
double ear rate % (DE)	0.00	0.50	0.16	0.15
setting percentage % (SP)	0.23	2.48	0.97	0.51
plant height (PH, cm)	269.85	328.82	306.18	16.24
ear height (EH, cm)	104.13	163.92	129.89	12.22
plant type (PT)	1.43	2.57	2.07	0.28
greenness retention (GR)	1.00	2.43	1.52	0.37
biological dry weight (DW, kg/hm ²)	14528.15	19818.13	16771.63	86.82



* p<0.05 ** p<0.01

Fig. 1. Phenotypic correlation coefficients among traits of silage maize (*Zea mays*) related to biological fresh weight yield. SP, setting percentage; DE, double ear rate; SL, stalk-lodging rate; PT, plant type; GR, greenness retention; PH, plant height; EH, ear height; FW, biological fresh weight; DW, biological dry weight. In the color gradient chart, the darker the blue color, the more significant the negative correlation; the darker the red color, the more significant the positive correlation; and when the color is colorless, it indicates no significant correlation.

Table 3. Feature Vectors and Contribution Rates of Principal Components of Each Trait Evaluated during an Investigation of Characteristics Associated with the Biological Fresh Weight of Silage Maize

Traits	Principal Components After Rotation			
	1	2	3	4
EH	0.860	0.076	0.049	0.223
GR	0.782	0.152	0.256	-0.257
PH	0.765	-0.045	-0.215	-0.188
DE	0.587	-0.302	-0.273	-0.068
FW	0.131	0.923	0.184	-0.007
DW	-0.166	0.877	0.160	-0.091
GP	-0.619	0.631	0.276	0.216
SP	0.120	0.625	-0.544	-0.145
PT	-0.043	0.294	0.807	-0.101
SL	-0.118	-0.067	-0.046	0.964
Eigenvalue	3.272	2.443	1.077	1.017
Contribution%	32.720	24.431	10.768	10.170
Accumulative Contribution%	32.720	57.151	67.919	78.089

Notes: setting percentage (SP), double ear rate (DE), stalk-lodging rate (SL), plant type (PT), greenness retention (GR), plant height (PH), ear height (EH, biological fresh weight (FW) and biological dry weight (DW)

Principal Component Analysis

Principal component analysis was performed on the 10 characteristics of silage maize, and the number of principal components was determined by examining eigenvalues greater than 1. The eigenvalues of the first 4 principal components were greater than 1; therefore, the original 10 correlated traits were converted into 4 new comprehensive traits, including most of the information on all investigated traits, where the cumulative contribution rate reached 78.089% (Table 3). The first principal component comprised EH, GR, and PH, accounting for 32.720% of the original data. The second principal component was composed of biological FW and biological DW, accounting for 24.431% of the original data. The third principal component comprised PT, which accounted for 10.768% of the original data. The fourth principal component comprised the SL rate, accounting for 10.170% of the original data.

Clustering Analysis

According to the aforementioned formulae, the comprehensive evaluation value (D) of the biological FW-related traits of silage maize at the maturity stage could be calculated. The 37 silage maize varieties were divided into 6 categories (Fig. 2). Category I contained two varieties (Chengdan 920 and Chengdan719) with low yield and high green retention. Category II contained two varieties (Mian 1902 and Nanqing 232) with a high lodging rate. Category III contained one variety (Nanqing 385) with the lowest biological FW and DW. Category IV contained nine varieties (e.g., Chengqing 385, Miandan 72, and Jishengyu 85) with the highest biological FW and DW. Category V contained 13 varieties (e.g., Nanqing 521, Chengdan 608, and Yayuqingzhu 8) with high biological FW and DW. Finally, category VI contained 10 varieties (e.g., Nanqing 2142, Chengdan 3601, and Chengdan 768) with high biological FW and the shortest GP.

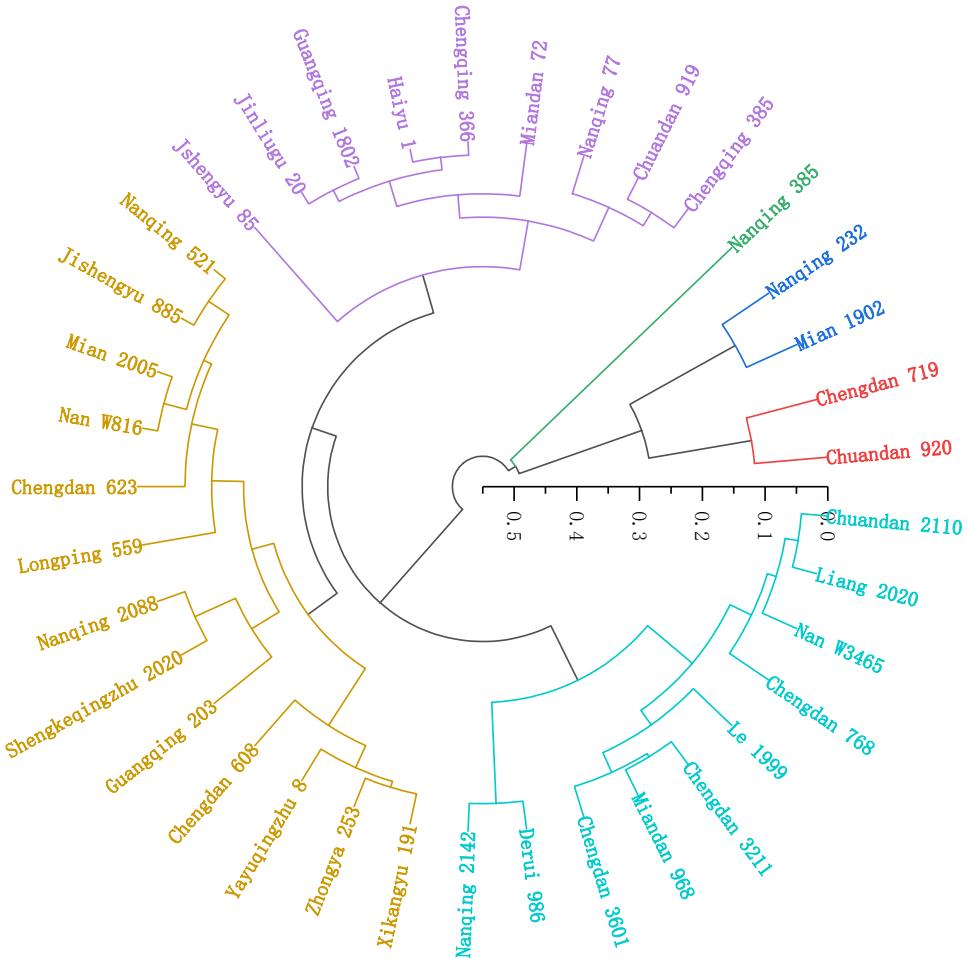


Fig. 2. Cluster of the 37 silage maize varieties investigated for characteristics related to biological fresh weight in southwestern China. Category I contains two silage maize varieties (Chengdan 920 and Chengdan 719) with low yield and high green retention. Category II contains two silage maize varieties (Mian 1902 and Nanqing 232) with a high lodging rate. Category III contains one silage maize variety (Nanqing 385) with the lowest biological FW and DW. Category IV contains nine silage maize varieties (e.g., Chengqing 385, Miandan 72, and Jishengyu 85) with the highest biological FW and DW. Category V contains 13 silage maize varieties (e.g., Nanqing 521, Chengdan 608, and Yayuqingzhu 8) with high biological FW and DW. Category VI contains 10 silage maize varieties (e.g., Nanqing 2142, Chengdan 3601, and Chengdan 768) with high biological FW and the shortest GP. FW, biological fresh weight; DW, biological dry weight; GP, growth period.

Discriminant Analysis

Discriminant analysis was used to verify the clustering results. Based on the clustering results, Fisher's linear discriminant function was obtained using the four principal components as discriminant variables.

$$S1 = -6.167 - 3.239x_1 - 3.438x_2 + 0.899x_3 \quad (4)$$

$$S2 = -5.314 - 3.433x_1 + 0.203x_2 + 0.103x_3 \quad (5)$$

$$S3 = -9.854 - 2.241x_1 - 3.082x_2 - 2.038x_3 \quad (6)$$

$$S4 = -5.478 - 0.715x_1 + 5.72x_2 - 0.051x_3 \quad (7)$$

$$S5 = -2.472 + 0.099x_1 - 2.385x_2 + 0.705x_3 \quad (8)$$

$$S6 = -4.379 + 2.074x_1 - 1.093x_2 - 0.868x_3 \quad (9)$$

The 37 varieties of maize silage were reclassified by discriminant analysis. As a result, two varieties in category V were reclassified into category II, one variety in category V was reclassified into category IV, one variety in category V was reclassified into category VI, and one variety in category VI was reclassified into category V. In summary, 32 varieties of maize silage were correctly identified, with a probability of judgment of 86.49%. Therefore, the 37 varieties of silage maize can be reliably classified into four groups according to biological FW and related characteristics.

Ridge Regression

To screen for comprehensive biological FW traits, a mathematical evaluation model was established to accurately evaluate the biological FW of the silage maize varieties. The D value was taken as the dependent variable, and the six main traits determining the principal component were considered independent variables for collinearity diagnosis. The SL rate trait had a significance level of $\alpha = 0.867 > 0.05$, and the PT trait had a significance level of $\alpha = 0.770 > 0.05$. Ridge regression analysis was performed on the other four traits, and the coefficients were selected as unstandardized coefficients. Finally, the regression equation was established as follows,

$$D = 0.206 \times EH + 0.205 \times GR + 0.725 \times DW - 0.138 \times PH \quad (10)$$

The equation's determination coefficient R^2 was 0.818; adjusted R^2 was 0.796, F was 36.026, and $p = 0.000 < 0.01$. The results showed that plant height, ear height, greenness retention, and biological DW had a significant linear relationship with the D value. The standardized data for the four traits were substituted into the equation to obtain the regression value. The root mean square error between the regression and original D values was calculated as 0.42. The regression value was in good agreement with the original D value, indicating that the regression equation established in this study had high accuracy and could be used to evaluate the biological FW of maize silage.

Boxplot

To show the high yield and stability of category IV silage maize varieties with the highest biological FW yield in various parts of southwest China, 2-year multi-point plots of the biological yields of nine maize varieties, which were constructed according to the cluster analysis results (Fig. 3). The average biological yield of seven silage maize varieties was maintained at 60,000 kg/hm², there was no significant difference within the group. Among these, Miandan 72 had a small fluctuation but was relatively stable, with good comprehensive performance and slightly higher yield, followed by Guangqing 1802 and Haiyu 1. In contrast, the biological FW yield of Chengdan 919 showed the worst performance.

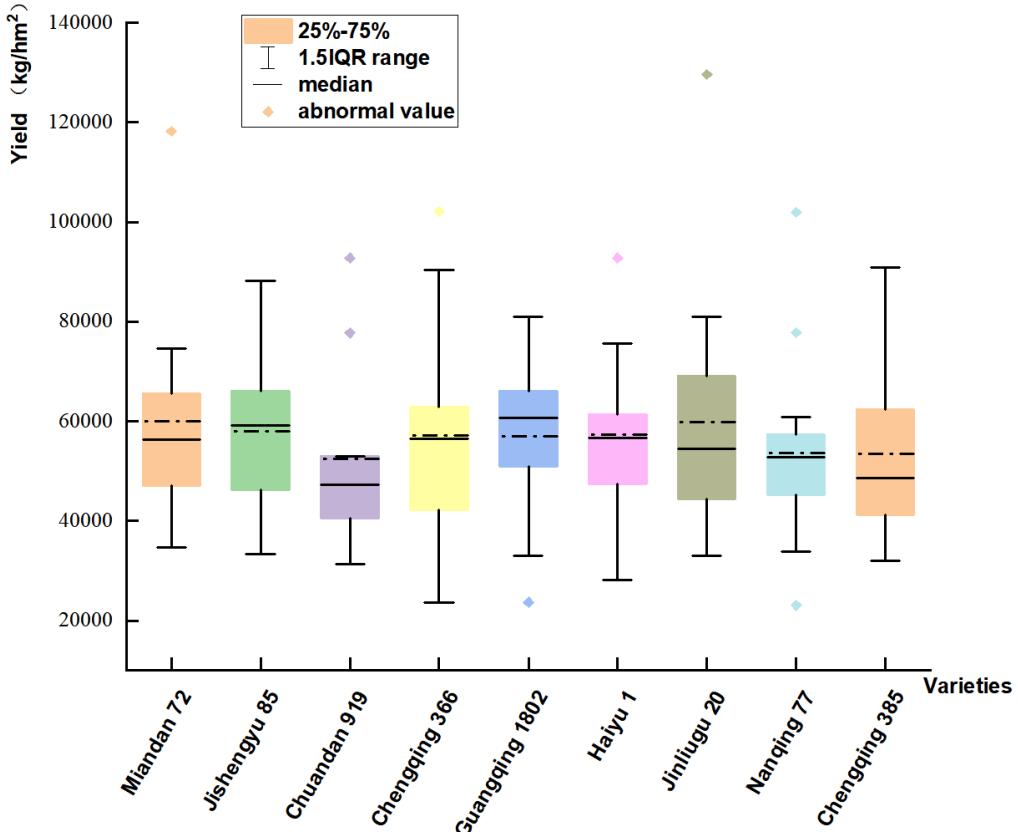


Fig. 3. High yield and stability of category IV silage maize varieties in various parts of Sichuan Province (* $p < = 0.05$, ** $p < = 0.01$)

Bioproduction-related Characteristics of Silage Maize at the Maturity Stage

Biological FW is strongly and positively correlated with GP, biological DW, PT, SP, and GR. This conclusion is consistent with those of previous reports. In production, the longer the GP of maize, the more dry matter accumulates and the larger the biological FW when there is little difference in water content (Tolera *et al.* 1998). Under high density, the more compact the leaf type, the higher the photosynthetic efficiency, and the greater the dry matter accumulation. In the present study, maize was planted at 60,000 plants/ hm^2 , and the more compact the maize silage, the higher its biological yield. GR is an important index for evaluating maize silage and is closely related to maize grains, and several sections of quantitative trait loci for GR overlap with yield quantitative trait loci (Zheng *et al.* 2010). Maize grain mass is the main component of aboveground mass, accounting for up to 50% (Bunting 1976). Therefore, a high empty-stalk ratio will significantly reduce the biological yield of maize silage. Similar to the present study, Viana *et al.* (2020) investigated key agronomic traits—including plant height, ear height, stalk diameter, planting density, husk coverage, cob number, unhusked ear weight, husked ear weight, and biological fresh weight yield—demonstrating their strong interrelationships. Notably, the authors highlighted robust correlations between maize biological yield and three critical traits: plant height, ear height, and unhusked ear weight. These findings align with the present observation that vertical growth parameters (*e.g.*, plant and ear height) and reproductive biomass (*e.g.*, unhusked ear weight) are pivotal drivers of silage maize productivity. Such consistency underscores the importance of integrating these traits into breeding programs aimed at optimizing yield components for forage applications.

Breeding silage maize hybrids requires balancing yield and quality (Pan *et al.* 2002). The biological FW of maize consists of stalks, ears, leaves, and tassels aboveground and is related to many factors. Through the analysis of various traits, many scholars have reported that female ear weight, PH, EH, GP, and biological yield of maize are highly correlated (Thomson and Rogers 1968; Donald and Hamblin 1976; Reminson and Akinleye 1978; Myoung *et al.* 2015). The biological yield of maize has also been proposed to positively correlate with the number of green leaves and chlorophyll content (Gitelson *et al.* 2014). In addition, stem diameter, internode number, and bract number are closely related to maize biological yield and a regression equation with silage maize biological yield as the dependent variable has been established (Liao *et al.* 2011). Compared with previous reports, the present study added correlations among SP, DE rate, SL rate, GR, PT, biological DW, and the biological FW of maize and comprehensively identified the factors affecting the biological FW of maize, providing a reference for the breeding of maize silage. Simultaneously, a mathematical evaluation model for the biological FW of silage maize was established based on PH, EH, GR, and biological DW, which could be used to evaluate the biological FW of silage maize.

Development Status of Hilly Silage Maize in Southwest China

In recent years, with the rapid development of animal husbandry in south China, there has been a large shortage of forage maize silage, contributing to the urgency to develop local maize silage. Ecological problems, such as lack of light, poor soil, high frequency of diseases and pests (Zhao *et al.* 2018), high temperature and humidity in summer (Li *et al.* 2018), and low temperature and dryness in winter, have long existed in southwest China (Ma *et al.* 2013). Fortunately, during the corn growing season in this region, rainfall ranges from 600 to 700 mm, which is conducive to robust maize growth. Under typical conditions, artificial irrigation is unnecessary. Maize can only be planted in one season, with serious diseases resulting in low yields. Silage maize requires a higher density and biological yield than grain maize. The planting density of maize silage in southwest China is 60,000 to 70,000 plants/hm², but the biological yield is only 42,000 to 68,000 kg/hm².

Key factors influencing yield include ecology, genetics, and agronomy. For example, the crop rotation pattern in the cultivation process can effectively reduce nitrogen loss, thus increasing the biological yield of maize (Komainda *et al.* 2017). With an increase in planting density, the biological yield of maize first increases and then decreases (Tang *et al.* 2018). While selecting stress-resilient corn varieties and increasing planting density can enhance maize biomass, these practices often compromise silage quality. Thus, optimizing cultivar selection and planting density is critical for sustainable silage maize production in China's Sichuan region (Zhao *et al.* 2022). Meanwhile, cultivation measures such as fertilization level (Harshbarger *et al.* 1954; Patni and Culley 1989) and harvest period (Giardini *et al.* 1976) greatly impact the yield of silage maize.

CONCLUSIONS

1. In the two-year trial in Nanchong, Sichuan, multivariate analysis was carried out to comprehensively evaluate the biological yield of 37 maize silage varieties. All 10 traits related to biological fresh weight were correlated. Principal component analysis showed that four principal component factors translated from these 10 traits accounted

for 78.1% of the original data. The four principal component factors include EH, GR, PH, FW, DW, PT, and SL rate. Therefore, in the process of selection and breeding, we should pay more attention to these several trait characteristics.

2. Cluster analysis divided the 37 maize silage varieties into six categories, and discriminant analysis indicated that 32 varieties were properly categorized. Ridge regression analysis selected four traits (plant height, ear height, greenness retention, and dry weight) to establish a yield evaluation model for maize silage. The use of predictive models helps in forecasting the biological yield of silage corn.
3. Principal component analysis was used to further classify silage maize. When screening maize varieties with high biological fresh weight, those with a long growth period, compact plant type, good green retention, and heavy dry weight can be selected.

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