Comparative Preliminary Evaluation of Agronomic and Quality Traits of New "Zhongtang" Superior Lines

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Abstract [**Objectives**] Sugarcane is the most significant sugar cash crop in the tropical and subtropical regions of China. However, it is notable for its narrow genetic basis, limited trait improvement, weak adaptability of varieties, and poor planting efficiency. In order to accelerate the process of sugarcane variety replacement and expand the basis of genetic variation, interspecific hybridization and multiple mutagenesis are the most effective methods for obtaining new sugarcane varieties. The evaluation and identification of phenotypic traits of germplasm represents a significant analytical method. The "Zhongtang" series of sugarcane varieties is distinguished by its high yield and high sugar content. New sugarcane germplasms with excellent agronomic and quality traits can be identified and developed as breeding parents and new breeding lines through comprehensive evaluation of the existing germplasm. [**Methods**] A total of 181 new lines were selected through genetic origin and orientation, and evaluated and appraised for growth, yield, and economic characteristics. The data on 6 important agronomic and quality traits, including tillering, initial plant height, plant height, stem diameter, number of effective stems, and brix at maturity, were used to discover superior lines. These traits were evaluated during the two production seasons of the participant lines. [**Results**] A comprehensive evaluation of seedling growth traits and economic traits at maturity of the experimental lines identified 30 excellent new germplasms of sugarcane. Among the selected lines, 1501 and 1701 exhibited superior agronomic and quality traits, rendering them suitable as parental lines for sugarcane breeding or breeding as new varieties. [**Conclusions**] The exemplary results obtained in this study provide a solid foundation for the improvement of sugarcane germplasm, with the goal of enhancing quality and efficiency. These findings are of great scientific and practical significance to the study of sugarcane yield and sugar-related genes, as well as the exploration of the evaluation and utilization of sugarcane germplasm resources.

Key words Sugarcane, Germplasm evaluation, Yield, Agronomic trait, Economic trait

1 Introduction

Sugarcane (*Saccharum officinarum* Linn.) is a perennial C4 high photosynthetic capacity plant that is widely planted as a major sugar crop in Guangxi, Yunnan, Guangdong, Hainan, and other provinces (regions) in China[1]. It is the most important sugar cash crop in tropical and subtropical regions of China[2]. Furthermore, sugarcane exhibits a high photosynthetic rate and dry matter accumulation capacity, coupled with an optimal energy yield ratio[3]. This makes it one of the most suitable bioenergy crops, with alternative energy sources mainly for ethanol and biomass production[4–5]. The stable development of the sugarcane industry plays a pivotal role in the growth of both the sugar and energy industries in China.

The main modern sugarcane varieties are from the interspecific crosses and backcrosses between tropical species (*S. officinarum* Linn., $2n = 80$) and wild species (*S. spontaneum* Linn., $2n = 40 - 128$). Nevertheless, the frequent use of few high-yielding and high-sugar tropical parents for backcrossing during the lengthy process of "noble" breeding has resulted in similar genetic relationships and the narrow genetic basis of modern sugarcane varieties[6]. Currently, 80% – 90% of the genome sequences of commonly sugarcane hybrid varieties are derived from *S. officinarum*[7–8]. This limits the potential for upgrading varietal traits, such as improving adaptability and increasing planting efficiency. Consequently, the enrichment of the genetic basis of sugarcane germplasm can provide a greater range of options for the genetic improvement of complex economic traits and facilitate the replacement of sugarcane varieties.

Among the genera of sugarcane, crosses between *S. officinarum* Linn. and *S. spontaneum* Linn. with Indian and Chinese species are more compatible and more suitable for expanding the genetic background of the "noble" tropical species of sugarcane. The application of mutagenesis breeding techniques based on spaceflight mutagenesis and radiation mutagenesis can enhance the mutation rate, expand the mutation spectrum, and facilitate the acquisition of mutation materials that are challenging to generate through interspecific hybridization[9]. Moreover, the majority of variants generated by induction are single-base variants, predominantly traits controlled by single genes with a main effect. Consequently, the target traits are stabilized rapidly, which can effectively reduce the breeding period and enhance the probability of breeding new varieties in subsequent screening and utilization[10].

Currently, research on sugarcane germplasm resources is primarily focused on identifying and characterizing phenotypic traits[11]. While molecular markers have been extensively utilized

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in the analysis of genetic diversity in sugarcane[12-16], phenotypic traits are straightforward and discernible, cost-effective and efficient, with expedient data collection, and closely related to production practice, which play an indispensable role in the analysis of genetic diversity in sugarcane[17]. A significant number of studies have been conducted to analyze the genetic diversity of phenotypic traits in sugarcane germplasm resources. Liu Xinlong *et al.*[18] conducted a genetic diversity analysis of 1 160 sugarcane germplasm resources using 17 qualitative traits and 5 quantitative traits. The results demonstrated that the quantitative genetic variation in the population of varieties from different origins exhibited considerable variability. The innovation of sugarcane germplasm was more active in three regions: the United States, China's Taiwan, and China, as well as Australia. The genetic variation of phenotypic traits in sugarcane variety populations primarily originated within the sources, with a significant genetic exchange occurring between the variety populations in different sources. Wu Jiantao *et al.*[11] conducted a genetic diversity analysis of 116 sugarcane germplasm resources using 35 phenotypic traits. Their findings revealed that the Yuetang series of sugarcane parents exhibited a high genetic diversity level, with a notable richness in leaf width and post-exposure stem color. Moreover, the Yuetang parents exhibited the highest diversity index in the 1980s. Zhao Yong *et al.*[19] evaluated 317 sugarcane germplasm resources using a rapid grading method for agronomic traits. The results demonstrated that plant height, stem diameter, effective stem, degree of leaf disease, and overall vigor were significant agronomic traits that reflected the field performance of sugarcane germplasm resources. Mao Jun *et al.*[20] employed a combination of phenotypic and molecular marker data to analyze 147 strains of asexual *Saccharum arundinaceum* germplasm. This analysis led to the identification of 16 superior materials, which were subsequently incorporated into the construction of *S. arundinaceum* microcore germplasm. Xu Chaohua *et al.*[21] utilized 162 *S. arundinaceum* germplasm resources to investigate their phenotypic traits and genetic diversity. Their findings revealed that the genetic diversity index of qualitative traits in *S. arundinaceum* germplasm resources was relatively low. Overall, the genetic diversity of *S. arundinaceum* germplasm resources was low, while the genetic variation of quantitative traits was more abundant. The genetic variation of the population primarily originated within the collection site, and there was a substantial genetic exchange between populations. Additionally, the genetic structure was not evidently differentiated.

In our preliminary studies, we conducted hybridization between wild resources of sugarcane, wild blood progeny, cultivated originals and commercial varieties through crossbreeding and spaceflight mutagenesis breeding. A "five-nursery system" of germplasm nursery, including parental nursery, seedling nursery, selection nursery, strain identification nursery and variety comparison nursery, had been established, containing progeny strains from 382 hybrid combinations. A mutant seedling nursery comprising approximately 7 000 seedlings were established through radiation and spaceflight mutagenesis of sugarcane seeds, seedlings, and tissue culture seedlings. Through the observation and screening of the growth characteristics, yield characteristics, comprehensive resistance, and adversity resistance of each strain of the new germplasm of sugarcane of hybrid breeding and mutation breeding, 380 germplasm strains rich in phenotypic variations and 204 superior lines were obtained. Finally, "Zhongtang" series of sugarcane varieties with high yield and high sugar content were selected and bred.

This study employed a comprehensive evaluation and analysis of 181 sugarcane lines with diverse sources of variation to assess their performance in key generative and technological traits, such as seedling growth characteristics, yield and economic characteristics at maturity. The goal was to identify sugarcane germplasm resources with excellent technological traits and cultivate new varieties of sugarcane that have multiple resistances, high yields, and high sugar. This would provide a reference for the genetic improvement of sugarcane economic traits and the innovative utilization of sugarcane germplasm.

2 Materials and methods

2.1 Materials

2.1.1 Experimental materials. A total of 181 sugarcane germplasm lines participated in the study, all of which were selected by the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, as intermediate materials for breeding. The germplasm lines were selected in 2020 from three distinct nurseries: the sugarcane hybrid selection nursery, the mutant seedling nursery, and the spaceflight seedling nursery. They were then subjected to standardized cultivation in the Hainan sugarcane experimental base of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, which is located in Huangtong Town, Lingao County, Hainan Province.

A total of 131 lines were selected from the hybrid selection nursery, derived from 25 hybrid combinations with "Zhongtang" varieties as the parent. The parental information of hybrid combinations is detailed in Table 1.

The 325 lines in the mutant seedling nursery were derived from the mutant progeny of Roc22 seedlings that had been subjected to ^{60}Co-γ radiation. The 230 lines in the spaceflight seed nursery were derived from the mutated progeny of sugarcane seeds that were launched on the Long March 5B carrier rocket in May 2020. In February 2021, all lines were subjected to field testing to ascertain their number of mature stems, plant height, stem diameter and stem central brix. The data from the control variety Roc22, grown in the same plot, was employed as a screening criterion to identify lines exhibiting three of the four sets of measurements at a level exceeding that of the control variety. For the control variety Roc22, the mean number of mature stems per 20 plants was 3.25, the mean plant height was 279.70 cm, the mean stem thickness was 26.42 mm, and the mean stem central brix was 17.60. A total of 50 lines were evaluated for agronomic and quality traits, comprising 25 radiation-induced and 25 spaceflight-induced lines, in addition to the hybrid lines.

Table 1 Parental details of hybrid combinations

No.	Female parent	Male parent	No.	Female parent	Male parent
1	Dezhe 07-36	Zhongtang 12-02	14	Yuetang 93-124	Zhongtang 16-02
2	Guitang 03-8	Zhongtang 13-01	15	Zhanzhe 50	Zhongtang 12-01
3	Guitang 04-1545	Zhongtang 12-02	16	Zhongtang 12-01	Guitang 02-761
4	Guitang 92-66	Zhongtang 15-01	17	Zhongtang 12-01	Guitang 92-66
5	Guitang 92-66	Zhongtang 12-01	18	Zhongtang 12-01	Yacheng 97-24
6	Ke 5	Zhongtang 13-01	19	Zhongtang 12-01	Yuetang 91-976
7	Liucheng 05-136	Zhongtang 12-01	20	Zhongtang 12-02	CP72-1210
8	Liucheng 05-136	Zhongtang 12-02	21	Zhongtang 12-02	Chuantang 89-103
9	Liucheng 07-536	Zhongtang 12-01	22	Zhongtang 12-02	Guitang 03-91
10	Reying 1	Yuetang 93-159	23	Zhongtang 12-02	Guitang 04-1545
11	Zhongtang 16-01	Guitang 02-761	24	Zhongtang 12-02	Yuetang 91-976
12	Yuetang 03-393	Zhongtang 12-02	25	Zhongtang 13-01	Roc10
13	Zhongtang 17-01	HoCP01-517			

2.1.2 Overview of the test site. The test site is situated in Huangtong Town, Lingao County, Hainan Province, which is subject to the tropical monsoon climate. The climate is characterized by high temperatures and high humidity, with a clayey, brick-red soil that is weakly acidic and of medium fertility. The previous crop was sugarcane.

2.2 Methods In March 2021, 181 sugarcane lines and the control variety Roc22 were planted on the same plot in the Hainan sugarcane experimental base. The experimental lines were arranged sequentially without repetition, with one plot for each line. One plot was planted in a single row, with 20 plants planted. The row length was 7 m, the row spacing was 1.2 m, and the plant spacing was 0.35 m. A protective row was set up around the perimeter. The management of field operations was conducted at a level of responsibility that was slightly more elevated than that of local management. All field management operations, including mid-tillage and soil cultivation, fertilizer application, drainage and irrigation, and pest control, were completed on the same day with the same technical measure.

The experiment was conducted over two consecutive production seasons, from 2021 to 2023, with the objective of measuring 6 traits in newly planted and ratoon sugarcane germplasm. These traits included tillering, initial plant height, plant height, stem diameter, number of effective stems, and stem central brix. In accordance with the methodology outlined in the *Sugarcane Germplasm Resources Description Specification and Data Standard*[22], the tillering rate and initial plant height of all experimental lines were subjected to investigated and enumerated with the objective of discerning the growth disparities exhibited by the evaluated lines. At the maturity stage, the plant height, stem diameter, and effective stem number of all experimental lines were investigated and counted in order to compare the yield differences between the evaluated lines. In December and January, the stem central brix of all the experimental lines was investigated and counted in order to facilitate a comparison of sugar differences between the evaluated lines.

2.3 Data processing The data were organized using the Microsoft Excel 2010 software, and the maximum, minimum, mean, standard deviation, and coefficient of variation of each trait were calculated. The data from two production seasons were averaged for the purpose of statistical analysis.

3 Results and analysis

3.1 Distribution of trait diversity among experimental germplasms As illustrated in Fig. 1, the 181 sugarcane lines exhibited a stem central brix range of 15.00 – 21.50, with an average of 17.28. Additionally, the number of mature stems per plant ranged from 1 to 15, with an average of 6.28. The plant height spanned 177.15 – 330.20 cm, with an average of 242.40 cm. Finally, the stem diameter ranged from 19.17 to 34.80 mm, with an average of 27.09 mm.

3.2 Comparative evaluation of growth traits of experimental germplasms Fig. 2 illustrates the range of tillering rates observed in the 181 sugarcane lines participating in the study, with values spanning from 0.90 to 4.10 and an average tillering rate of 2.38. The tillering rate of the control variety, Roc22, was 2.40. Seventy-six experimental lines exhibited a tillering rate that exceeded that of the control Roc22. Of these, 42 lines exhibited a tillering rate more than 3, representing a proportion of 25% more of the control. Fourteen experimental lines exhibited a tillering rate that was less than 60% of that of Roc22. Of these, 6 lines exhibited a high number of withered heart seedlings, which were insufficient tillers due to stem borer infestation. The remaining 8 lines exhibited weak growth and an insufficient tillering rate.

The initial height of the 181 sugarcane lines exhibited a considerable range, with a mean of 139.06 cm. The minimum height was 86.40 cm, while the maximum was 183.60 cm. The initial height of the control variety Roc22 was 123.64 cm. The tillering rate of the 138 tested strains exceeded that of the control Roc22, and the initial plant height of 39 strains reached more than 154.60 cm, which exceeded the initial plant height of Roc22 by 25%. Eleven of the experimental lines exhibited initial plant heights that were less than 85% of Roc22. Four of these lines exhibited similar undergrowth due to a high number of withered hearted seedlings at the seedling stage, which were attributed to stem borer infestation. The remaining 7 lines exhibited weak growth potential.

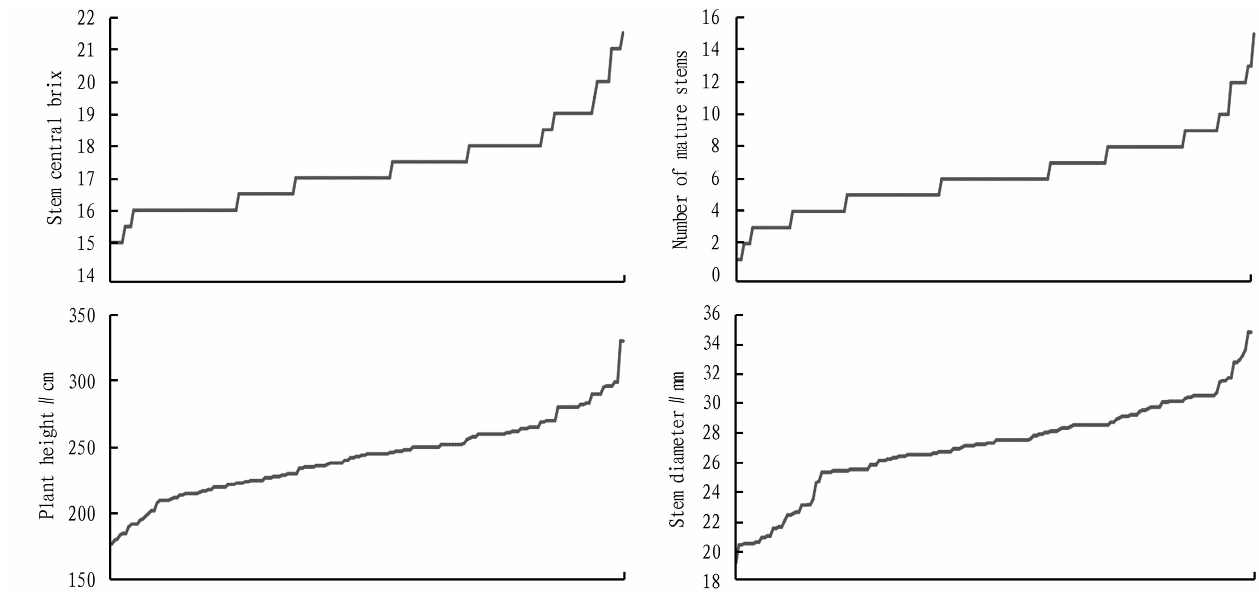
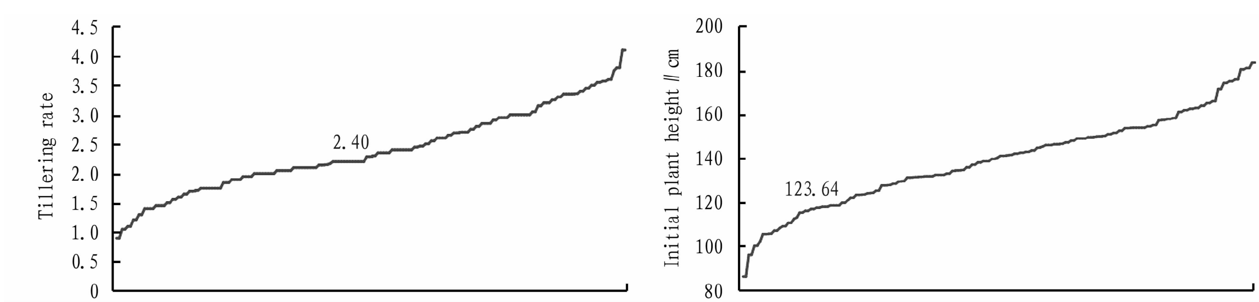


Fig. 1 Distribution of trait diversity in experimental germplasms



NOTE Tillering rate and initial plant height of the control variety Roc22 are labeled in the figure.

Fig. 2 Comparative evaluation of growth traits of experimental germplasms

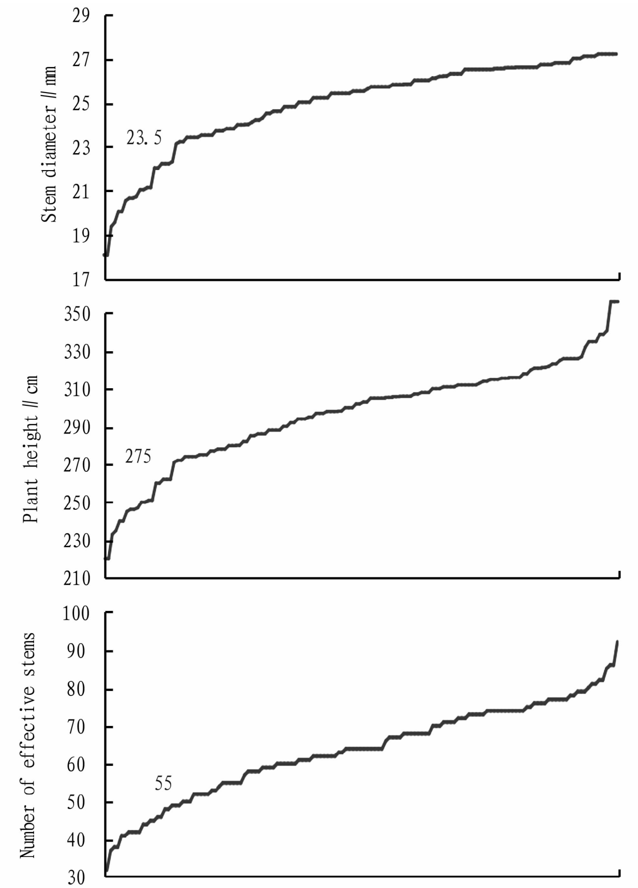
A comprehensive comparison of seedling growth traits revealed that 6 lines exhibited a higher prevalence of withered heart seedlings and more borer damage. The surveys at the seedling and tillering stage revealed that 19 lines exhibited heavy to top rot and mosaic disease. In the survey conducted at the jointing stage, 17 lines were found to be severely afflicted by smut.

3.3 Comparative evaluation of yield traits of experimental germplasms Stem diameter, plant height and number of effective stems at maturity of sugarcane are crucial yield components. They were utilized to assess the yield of the experimental lines in this study.

The plant height of the 181 lines exhibited a range of 220.00 to 356.00 cm, with a mean height of 296.78 cm. The control variety Roc22 exhibited a plant height of 275.00 cm, while 113 lines exhibited heights exceeding that of the control variety. Notably, only 3 lines reached a height of more than 345.00 cm, which was more than 25% of the height of Roc22. Only 2 lines exhibited a height that was less than 80% of that of Roc22. The overall plant height of the experimental lines was considerable.

Fig. 3 illustrates the range of stem diameters observed in the 181 lines, which spanned from 18.00 to 31.60 mm, with an average stem diameter of 25.68 mm. The stem diameter of Roc22 was 23.50 mm. The stem diameter of 113 experimental lines exceeded that of the control variety. Nine of these lines had a stem diameter of 29.50 mm or more, exceeding 25% of Roc22. These lines were identified as large-stemmed varieties. Only two of the experimental lines exhibited stem diameters that were less than 80% of that of Roc22. The stem diameters of the experimental lines exhibited greater uniformity, with the majority of them exhibiting medium to large stem sizes.

The variation in effective stem number among the 181 lines surveyed ranged from 32.00 to 92.00, with an average effective stem number of 63.61. The effective stem number of the control variety Roc22 was 55. The effective stem number of 110 test lines exceeded that of Roc22, and 52 lines reaching more than 70, representing a 27% increase over that of Roc22. A total of 12 experimental lines exhibited a stem number that was less than 80% of Roc22. The effective stem number of the experimental lines exhibited considerable variability. Furthermore, the effective stem number of the lines with a high tillering rate in the early stages was not necessarily indicative of a high number of effective stems at the final maturity stage. Some lines exhibited disease symptoms, including smut, during the middle and late stages of growth. This resulted in a reduction in the rate of stem formation and effect on the number of effective stems at the final maturity stage.



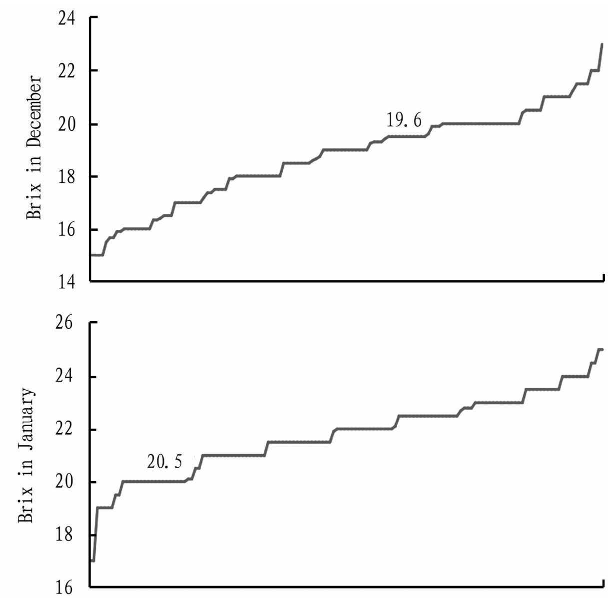
NOTE Stem diameter, plant height and number of effective stems of the control variety Roc22 are labeled in the figure.

Fig. 3 Comparative of yield traits of experimental germplasms

3.4 Comparative evaluation of sugar traits of experimental germplasms Fig. 4 illustrates that the stem central brix of the 181 lines observed at maturity stage was detected on two occasions. In the Hainan sugarcane area, the crushing process typically commences in December and concludes at the end of February in the following year. In this study, the stem central brix detected in December and January of the following year was employed to assess the sucrose content and sugar quality traits of the experimental lines.

In December, the variation in stem central brix of experimental lines ranged from 15.00 to 23.00, with an average brix of 18.74. The brix of the control variety, Roc22, was 19.60, and 48 experimental lines exhibited the brix exceeding that of Roc22. Conversely, only 4 lines exhibited the brix of 22.00 or more, which were more than 2% higher than the brix of Roc22. Nevertheless, the brix of the 38 lines was found to be 2% lower than that of Roc22. The range of stem central brix observed in the experimental lines in January spanned from 17.00 to 25.00, with an average of 21.77. The brix of Roc22 was 20.50, while the brix of 111 lines exceeded that of Roc22. Forty lines exhibited a brix of 22.50 or higher, which represented a 2% increase over the brix of Roc22. Twelve lines exhibited a brix of 24 or higher. A total of only 2 lines exhibited a brix below 18.5, representing a 2% reduction in brix compared to Roc22.

In general, the stem central brix of the experimental lines was higher in January than in December. This was due to a longer growing period and sugar accumulation. However, maturity periods of the experimental lines is different, the peak sugar content was reached at the earlier stage in the early maturing lines, while the peak sugar content in the middle and late maturing lines took a longer growing time to reach. Furthermore, the stem central brix of some late-maturing lines could be higher if the test was continued. Concurrently, the proliferation of early-maturing lines subsequent to maturity results in the depletion of sucrose content accumulation which can even extend to the reproductive growth of pregnancy and flowering. These growth processes then lead to the catabolism of sucrose content and a subsequent decrease in stem central brix.



NOTE Field brix of the control variety Roc22 are labeled in the figure.

Fig. 4 Comparative evaluation of sugar traits of experimental germplasms

3.5 Excavation of excellent germplasms The investigation and statistical analysis of two production seasons with the comprehensive evaluation of growth traits and economic traits at the seedling and mature stages, led to the selection of 30 new germplasms that exhibited excellent characteristics. The results are presented in Table 2. A comprehensive evaluation of the 30 Zhongtang lines revealed a rich genetic variation basis and a number of desirable agronomic and quality traits. These lines could therefore be used as sugarcane breeding parents and the basis for new varieties.

Among the lines, 4 lines had a plant height of more than 320.00 cm, including 1-3, 1501, 7-3, and 1701. Seven lines had a stem diameter more than 27.00 mm, including 1501, 4-6, 11-3, 2-1, 4-7, 6-1, and 1701. These lines have a high yield and can be utilized as high-yielding parents. Eight lines, including 19-9, 21-3, 1508, 1702, 4-9, 22-3, 2-2 and 4-7, had a high sucrose content of more than 17.00%, and thus represent potential candidates for utilization as high-sugar parents. The advantages of both yield and sucrose content were evident in 1501 and 1701, which can be cultivated as excellent new varieties.

Table 2 Excellent agronomic and quality traits of selected germplasms

No.	Line No.	Plant height // cm	Stem diameter // mm	Sucrose content // %
1	6-3	307.60	23.20	15.34
2	22-7	273.60	20.62	13.83
3	7-17	311.60	23.24	16.80
4	2-1	306.55	27.67	16.63
5	19-9	310.80	24.55	17.21
6	6-11	279.33	20.00	15.54
7	7-3	321.20	19.30	15.84
8	1-3	320.00	21.15	15.30
9	4-9	296.20	23.55	17.83
10	4-7	269.80	27.69	19.35
11	8-4	288.20	23.43	16.20
12	22-3	290.64	24.82	17.91
13	20-9	308.23	26.04	15.64
14	6-1	270.00	27.83	14.63
15	21-3	282.27	21.13	17.31
16	19-2	307.65	26.33	14.90
17	11-3	284.43	27.56	16.22
18	19-12	281.86	26.29	15.76
19	21-9	279.67	25.74	13.63
20	1-4	297.43	24.89	12.94
21	8-6	289.62	23.12	15.32
22	4-6	296.34	27.19	14.75
23	2-2	287.62	20.77	18.60
24	8-1	287.80	25.72	16.53
25	1702	313.41	26.22	17.42
26	1703	276.80	25.82	15.68
27	1501	321.08	27.10	15.39
28	1506	279.33	26.65	13.92
29	1508	287.61	23.40	17.34
30	1701	342.85	28.14	15.85

4 Conclusions and discussion

The exploration and utilization of sugarcane germplasm resources is paramount importance for the selection of breakthrough varieties and the genetic improvement of complex traits. The most basic method for achieving this goal is through the identification and evaluation of phenotypic traits. This represents a crucial basis for selecting parents in sugarcane hybrid breeding. The selection of parents with desirable comprehensive traits represents a fundamental principle for sugarcane hybrid breeding[23]. The objective of this study was to identify superior germplasm resources with high-quality performance as parents and superior varieties through comprehensive evaluation and analysis of 181 superior lines for agronomic and quality traits.

In this study, 30 sugarcane germplasms were screened for their agronomic and yield traits through comprehensive assessments of the growth, yield, and quality traits of new sugarcane lines. Among them, the new hybrid line created by the "Zhongtang" variety as the parent retains the characteristics of high yield and high sugar content. The mutant lines obtained by radiation mutagenesis and spaceflight mutagenesis were found to exhibit numerous resistance-related mutant phenotypes, including leaf sheath hair clusters and leaf margin serrations. However, these lines generally retained yield and sugar from the parent lines. The 30 superior lines can be utilized in the breeding of novel sugarcane varieties exhibiting multiple resistances, high yields, and high sugar contents.

Among the tested varieties, the superior line 1501 demonstrated noteworthy performance in the 15^{th} round of the national joint district test of sugarcane varieties. It has been selected for the official test of 13 district pilots, indicating its potential for wider dissemination and practical application. 1701 is a superior ratooning variety among the experimental lines, offering enhanced machinability and yield advantages. In conjunction with the agro-mechanical and agro-technical support for mechanized production, it has significant scientific and practical research implications for the improvement of sugarcane germplasm, with the objective of enhancing quality and efficiency, as well as research on genes related to sugarcane yield and sugar content.

Currently, the advancement of sugarcane breeding for high resistance in China is progressing at a gradual pace. Previous breeding efforts have emphasized high-yield and high-sugar breeding, while neglecting the source of genetic basis and comprehensive resistance enhancement. This has resulted in limited genetic variation in the parental population and the inability to obtain breakthrough varieties[24]. Therefore, it is necessary to carry out varietal breeding on the basis of expanding the genetic variation. The analysis of the phenotypic traits of "Zhongtang" sugarcane germplasm in this study is inherently subjective, and the evaluation of phenotypic traits is susceptible to being influenced by environmental factors. Consequently, the subsequent phase of the study entails the comprehensive genetic diversity analysis and trait mining of the superior lines that have been identified. This will facilitate the revelation of their intrinsic genetic information, the enhancement of resource utilization efficiency, and the provision of more effective services for the selection and breeding of sugarcane varieties with high superiority and multi-resistance.

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