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Comprehensive analysis for herbicide phytotoxicity and tolerance of sugarcane in China

Research Article

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Abstract

Weeds significantly reduce sugarcane (*Saccharum officinarum* L.) production by 30% to 50% and cause complete crop loss in severe cases. Guangxi, a central sugarcane-growing region in southern China, faces significant challenges due to the proliferation of weeds severely impacting crop tillering, yield, and quality. In this study, we surveyed and identified 35 weed species belonging to 16 families in Longzhou, Nongqin, and Qufeng, with significant threats posed by purple nutsedge (*Cyperus rotundus* L.), bermudagrass [*Cynodon dactylon* (L.) Pers.], hairy crabgrass [*Digitaria sanguinalis* (L.) Scop.], black nightshade (*Solanum nigrum* L.), white-edge morningglory [*Ipomoea nil* (L.) Roth], and ivy woodrose [*Merremia hederacea* (Burm. f.) Hallier f.]. The application of 81% MCPA-ametryn-diuron achieved greater than 90% control within 15 d. Although herbicides are effective, they can unintentionally harm sugarcane, indicating a need for tolerant genotypes. Therefore, we comprehensively evaluated herbicide-induced phytotoxic responses and identified tolerant sugarcane genotypes over 3 yr of trials conducted on 222 genotypes across Guangxi. We quantified phytotoxicity by counting the number and severity of affected leaves. The ANOVA revealed statistically significant main and interaction effects among genotype, crop cycle, and location. Cluster and discriminant analyses classified the genotypes into five groups: 21 highly tolerant (HT), 68 tolerant, 75 moderately tolerant, 18 susceptible, and 40 highly susceptible. The 21 HT genotypes demonstrated strong potential to be used as parental lines for breeding herbicide-tolerant varieties, to inform precision breeding strategies, and to increase tolerance to herbicide stress in sugarcane.

Introduction

Sugarcane (*Saccharum officinarum* L.) is a vital crop for sugar and energy production in China, with Guangxi being the primary cultivation region. According to the National Bureau of Statistics, Guangxi's sugarcane planting area has remained at more than 75 million ha, with sugar production around 6 million Mg (1000 kg) in recent years (Liu et al. 2023). High precipitation and warm temperatures provide ideal conditions for sugarcane cultivation. However, these conditions also support the growth of diverse weed species, significantly constraining sugarcane yields in Guangxi. Weed infestations can cause 20% to 30% yield reductions, and in severe cases, losses might exceed 50%, rendering fields unproductive (Li 2023; Lu et al. 2011).

Weed species composition in Guangxi sugarcane fields indicates low variation across the region, with most species belonging to subtropical families and smaller proportions from tropical and temperate zones (Sun et al. 2019). The predominant species include hairy crabgrass [*Digitaria sanguinalis* (L.) Scop.], Indian goosegrass [*Eleusine indica* (L.) Gaertn.], bermudagrass [*Cynodon dactylon* (L.)], knotgrass (*Paspalum distichum* L.), Canada thistle [*Cirsium arvense* (L). Scop.], stickywilly (*Galium aparine* L.), yellow foxtail [*Setaria viridis* (L.) P. Beauv.], hyssop (*Hyssopus officinalis* L.), and purple nutsedge (*Cyperus rotundus* L.) (Li et al. 2016). The Gramineae and Compositae families are the most diverse, with Gramineae weeds being the most harmful, followed by broadleaf weeds and less detrimental Cyperaceae weeds (Li 2023). Effective and timely weed management is essential for maintaining and improving sugarcane production.

Chemical herbicides play a pivotal role in weed control in sugarcane fields, particularly as the rising costs of rural labor have increased reliance on herbicides. More than 80% of sugarcane fields in Guangxi use herbicides for weed management (Guan et al. 2015). MCPA-ametryn-diuron (MAD), a formulation commonly used to control weeds, combines systemic and contact herbicidal effects (Lima et al. 2017; Rangani et al. 2022; Sun et al. 2021). Ametryn (a triazine herbicide) and diuron (a phenylurea herbicide) inhibit photosynthesis by disrupting electron transfer in photosystem II. MCPA-sodium (a phenoxy carboxylic acid hormone herbicide) interferes with plant hormone functions, leading to uncontrolled growth and death of broadleaf weeds (Negri et al. 2015; Zhang et al. 2022). A weed control efficacy of 87.9% against *C. rotundus* was reportedly achieved within 15 d by applying a 65% MAD formulation at 2,047.5 g ha^{-1} in 675 kg of water (Li et al. 2016). The 81% MAD wettable powder, composed of low-toxicity raw materials, meets national standards for efficiency, low toxicity, and environmental sustainability (Huo et al. 2018). Its rapid absorption through roots, stems, and leaves effectively controls a broad spectrum of annual and perennial weeds, making it a typical solution for sugarcane weed management. This formulation has yielded an annual economic benefit of 1.87 million yuan in recent years (Huang et al. 2015).

Using herbicides is currently the most efficient and time-saving approach for weed management in sugarcane cultivation. However, herbicides can lead to abnormal growth and development in sugarcane, resulting in varying degrees of damage that can negatively impact yield and quality (Hassan et al. 2023; Martins-Gomes et al. 2022; Wang et al. 2022). Developing and cultivating herbicide-tolerant varieties is an economical, environmentally sustainable, and effective strategy to mitigate herbicide-induced damage (Abou-Khater et al. 2022). Therefore, tolerant genotypes are essential to ensure sustainable and cost-effective sugarcane production. Globally, researchers are working to identify tolerant varieties to enhance the efficiency of herbicide management (Koutouan et al. 2023). Moreover, the variability in herbicide effects on sugarcane poses challenges for accurate identification and monitoring, emphasizing the need for robust classification standards and evaluation frameworks (Singh et al. 2024). However, research on herbicide-tolerant breeding and evaluation in sugarcane in China remains limited (Cheng et al. 2022; Su et al. 2022). This study aimed to establish a robust classification standard for herbicide tolerance of sugarcane genotypes, develop a comprehensive evaluation framework to assess sugarcane tolerance to these chemicals, and identify tolerant germplasm resources. The findings will enable the diagnosis and prediction of herbicide-induced sugarcane phytotoxicity, promoting sustainable production and advancing the sugarcane industry in China.

Materials and Methods

Experimental Sites

Field experiments were conducted at the Longzhou, Nongqin, and Qufeng Agricultural Experimental Stations in Guangxi, China, from 2021 to 2023. The soil type in all three experiments was red loam with sugarcane as the preceding crop. The Longzhou site is located at 22.3333°N, 106.7833°E, at an elevation of 115.4 m above sea level. The region has a subtropical monsoon climate characterized by an average annual temperature of 22.2 °C, a frost-free period of approximately 350 d, and an average annual precipitation of 1,300 mm. The area gets a mean annual sunshine duration of 1,695.2 h and a total solar radiation of 107.5 kcal cm^{-2}. The second and third experimental sites, Nongqin and Qufeng, are located in Fusui County (22.6418°N, 107.9191°E) at an elevation of 69.5 m above sea level. This area receives a mean annual sunshine duration of 1,693 h and a total solar radiation of 108.4 kcal cm^{-2}. The climate in Fusui features an average frost-free period of 346 d, annual rainfall ranging from 1,050 to 1,300 mm, and an average annual temperature of 22.4 °C (Supplementary Figure S1).

Weed Survey in the Sugarcane Field

The weed populations were surveyed using the W9 inverted point sampling method (Supplementary Figure S2) in the sugarcane fields at Longzhou, Nongqin, and Qufeng. Nine sampling sites were examined within each test plot, covering an area of 0.25 m^2. Weed assessments were conducted three times at each sampling point before herbicide application and on day 7 and day 15 after application. The collected data were then used to calculate the plant control efficiency using the following formula:

$$\text{Plant control efficiency (\%)} = \frac{\text{CK} - \text{PT}}{\text{CK}} \times 100 \qquad (1)$$

where CK represents the number of weed plants in the control area, and PT represents the number of weed plants in the treatment area.

Herbicide Application

An ADJB-20 knapsack sprayer (Yongxing Machinery, Hebei Province, China) equipped with a fan-shaped nozzle and a constant-pressure valve was used for herbicide application of the 81% MAD wettable powder supplied by Shandong Shengbang Luye Chemical Co., Ltd. (Weifang City, Shandong Province, China) at an average flow rate of 830 ml min^{-1}. The application rates of herbicide and water consumption per m^2 were 0.84 g and 90 g, respectively, with an additional 2.5 g of auxiliary agent per m^2. The treatments were applied when the sugarcane plants were at either the 5-leaf stage (seedling stage) or the 9-leaf stage (tillering stage). The herbicide was sprayed uniformly and vertically using the backpack sprayer before the closure of the sugarcane canopy. To ensure maximum effectiveness, the applications were scheduled to avoid rainfall within 6 h after spraying. A non-treated control was included for comparison.

Experimental Design and Field Management

The experiment was carried out in a randomized complete block design with three replications per genotype. Sugarcane genotypes were first planted at Nongqin and Longzhou in March 2021. The plants were maintained for 3 yr, which included 1 yr of new planting (2021), followed by 2 yr of ratoon cropping (2022 to 2023). Each genotype was planted in a single row per replication, with 2-m-long rows spaced 1.3 m apart at a planting density of 60 shoots per row. In March 2023, the identical 222 genotypes were planted at Qufeng and maintained through the first ratoon cropping cycle in 2024. At this site, the rows were 3-m-long, spaced 2 m apart, and planted at a density of 80 shoots per row, with three replications per genotype. To ensure consistency, fertilizer management practices were standardized across all sites, following local commercial sugarcane production methods. Additionally, the genotypes YT71-210 and ZZ6 were included at all locations as controls, representing susceptible and tolerant standards, respectively.

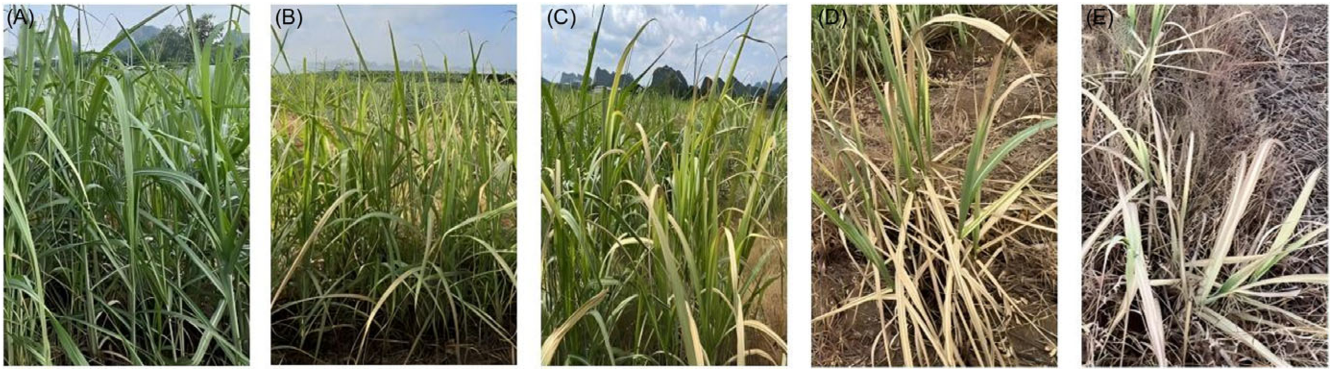

Figure 1. Symptom of 81% MCPA-ametryn-diuron (MAD) phytotoxicity on sugarcane. (A) level 0; (B) level 1; (C) level 2; (D) level 3; (E) level 4.

Field Evaluation

Field data were systematically collected over 3 yr (2021 to 2023) at the Longzhou site, 2 yr (2022 to 2023) at the Nongqin site, and 1 yr (2023) at the Qufeng site. For each genotype, we recorded the total number of plants (*N*), the number of plants exhibiting herbicide phytotoxicity (*m*), and the severity of phytotoxicity during the seedling stage (May to July). Phytotoxic severity was evaluated using a visual scale from 0 to 4 based on the symptoms observed on sugarcane leaves (Figure 1).

- **Level 0:** No visible herbicide injury; plants exhibited normal growth.
- **Level 1:** Mild symptoms, such as temporary yellowing at leaf tips and small damaged spots, which recovered quickly without affecting plant growth.
- **Level 2:** Moderate yellowing on fewer than half of the leaves, with continuous damage, chlorosis, and slight growth inhibition. Recovery was achievable, and yield remained unaffected.
- **Level 3:** Severe yellowing and drying on more than half of the leaves, accompanied by stunted plant growth, resulted in significant yield reductions and partial plant mortality, making recovery challenging.
- **Level 4:** Extensive yellowing and leaf death, severe growth suppression, widespread plant mortality, and substantial yield losses, potentially leading to complete crop failure.

Data from all three sites were combined to calculate the herbicide phytotoxic index, comprehensively assessing genotype responses under varying field conditions.

The herbicide phytotoxic percentage was calculated using the following formula:

$$Q = \frac{m}{N} \times 100 \tag{2}$$

where *Q* represents herbicide phytotoxic percentage (%), *m* denotes the number of plants exhibiting herbicide phytotoxicity, and *N* represents the total number of plants observed.

Depending on phytotoxic severity, the herbicide phytotoxic index was calculated using the following formula:

$$\mathrm{PI} = \frac{\sum (n \times s)}{S \times N} \times 100 \tag{3}$$

where PI denotes herbicide phytotoxic index, *n* represents the number of plants at each phytotoxic severity level, *s* denotes the assigned value for the severity grade, *S* indicates the highest possible severity grade, and *N* represents the total number of plants observed.

These calculations provided a comprehensive measure of herbicide impact on sugarcane across the three experimental sites.

Statistical Analysis

The weed control efficiency data from Longzhou, Nongqin, and Qufeng were processed and analyzed using Data Processing System software (v. 7.05, Zhejiang University, Hangzhou, China). An arcsine square-root transformation was applied to standardize the percentage of herbicide phytotoxicity across 222 sugarcane genotypes. The data were subsequently analyzed using ANOVA in R software (v. 3.5.0; Verma et al. 2022) to assess variation among genotypes, crop cycles, and locations. Specifically, a one-way ANOVA was performed on data from Longzhou and Nongqin to evaluate variations across different crop cycles. The model clearly defines nesting relationships and interactions to address data imbalance across various locations and times. Data were analyzed using a mixed linear model as follows:

$$Y_{ijklm} = u + C_i + G_j + L_k + Y_l(L_k) + R_m(L_k) + (G \times L)_{jk} + (G \times C)_{ji} + (G \times L \times C)_{jki} + E_{ijklm} \tag{4}$$

where Y_{ijklm} is the herbicide phytotoxicity percentage of the *j*th genotype from the *m*th replication in the *k*th location of the *l*th year of the *i*th crop cycle; *u* is the overall mean; C_i is the fixed effect of the *i*th crop cycle; G_j is the random effect of the *j*th genotype; L_k is the fixed effect of the *k*th location; $Y_l(L_k)$ is the *l*th year nested within the *k*th location; $R_m(L_k)$ is the *m*th replication nested within the *k*th location; $(G \times L)_{jk}$ is the interaction effect of the *j*th genotype and the *k*th location; $(G \times C)_{ji}$ is the interaction effect of the *j*th genotype and the *i*th crop cycle; $(G \times L \times C)_{jki}$ is the interaction effect of the *j*th genotype and the *k*th location and the *i*th crop cycle; E_{ijklm} is the experimental residual error.

Broad-sense heritability (H^2_B), defined as the proportion of phenotypic variance attributable to genetic variance, was estimated for individual years and through a combined analysis across multiple years and locations. The formula used for the combined analysis is as follows:

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Table 1. Investigation on weed control efficacy in sugarcane fields from Longzhou, Nongqin, and Qufeng, China.

		Weed control efficacy at 7 d (%)			Weed control efficacy at 15 d (%)			
Subject	Weed species	Longzhou	Nongqin	Qufeng	Longzhou	Nongqin	Qufeng	Lifestyle
Gramineae	*Echinochloa crus-galli* (L.) P. Beauv.	66.67	—	—	100.00	—	—	Annual herbs
	Eleusine indica (L.) Gaertn.	0.00	—	—	66.67	—	—	Annual herbs
	Digitaria sanguinalis (L.) Scop.	27.59	28.43	100.00	100.00	100.00	100.00	Annual herbs
	Cynodon dactylon (L.) Pers.	100.00	43.24	100.00	100.00	88.07	100.00	Perennial herbs
Asteraceae	*Bidens pilosa* L.	0.00	0.00	—	100.00	100.00	—	Annual herbs
	Youngia japonica (L.) DC.	100.00	100.00	—	100.00	100.00	—	Annual herbs
	Gamochaeta pensylvanica (Willd.) Cabrera	66.67	—	100.00	100.00	—	100.00	Annual herbs
	Erigeron acris L.	—	0.00	—	—	100.00	—	Biennial herbs
	Ageratum conyzoides L.	—	0.00	—	—	100.00	—	Annual herbs
	Blumea balsamifera (L.) DC.	—	0.00	—	—	100.00	—	Perennial herbs
	Gynura segetum (Lour.) Merr.	—	0.00	—	—	100.00	—	Perennial herbs
	Sonchus oleraceus L.	—	—	100.00	—	—	100.00	Annual or Biennial herbs
	Hemistepta lyrata (Bunge) Bunge	—	—	100.00	—	—	100.00	Annual herbs
	Youngia heterophylla (Hemsl.) Babc. & Stebbins	—	—	100.00	—	—	100.00	Annual or Biennial herbs
Convolvulaceae	*Ipomoea biflora* (L.) Pers.	100.00	—	—	100.00	—	—	Annual herbs
	Merremia hederacea (Burm. f.) Hallier f.	91.67	93.33	100.00	100.00	100.00	100.00	Annual herbs
	Ipomoea nil (L.) Roth	—	100.00	100.00	—	100.00	100.00	Annual herbs
Cyperaceae	*Cyperus rotundus* L.	26.14	36.26	94.93	95.45	90.53	96.77	Perennial herbs
Oxalidaceae	*Oxalis corniculata* L.	100.00	—	100.00	100.00	—	100.00	Perennial herbs
Solanaceae	*Solanum nigrum* L.	0.00	21.43	100.00	100.00	100.00	100.00	Annual herbs
Commelinaceae	*Commelina benghalensis* L.	100.00	—	—	100.00	—	—	Perennial herbs
Rubiaceae	*Spermacoce alata* Aubl.	100.00	82.01	—	100.00	100.00	—	Perennial herbs
Malvaceae	*Urena lobata* L.	—	92.86	—	—	100.00	—	Perennial herbs
Fabaceae	*Senna occidentalis* (L.) Link	—	100.00	—	—	100.00	—	Perennial herbs
	Gueldenstaedtia verna (Georgi) Boriss.	—	—	100.00	—	—	100.00	Perennial herbs
	Dunbaria villosa (Thunb.) Makino	—	—	100.00	—	—	100.00	Perennial herbs
Cucurbitaceae	*Cucumis melo* L.	—	72.73	—	—	100.00	—	Annual herbs
Polygonaceae	*Polygonum perfoliatum* L.	—	75.00	100	—	100.00	100	Annual herbs
	Persicaria maculosa Gray	—	—	100	—	—	100	Annual herbs
	Polygonum plebeium R. Br.	—	—	100	—	—	100	Annual herbs
Molluginaceae	*Trigastrotheca stricta* (L.) Thulin	—	33.33	—	—	100.00	—	Annual herbs
Sapindaceae	*Koelreuteria paniculata* Laxm.	—	0.00	—	—	100.00	—	Perennial herbs
	Cardiospermum halicacabum L.	—	—	100	—	—	100	Annual herbs
Caryophyllaceae	*Stellaria dichotoma* L.	—	—	100	—	—	100	Perennial herbs
Apiaceae	*Centella asiatica* (L.) Urb.	—	—	100	—	—	100	Perennial herbs

$$H^2{}_B = \sigma^2{}_g / (\sigma^2{}_g + \sigma^2{}_{gc}/c + \sigma^2{}_{gl}/l + \sigma^2{}_{glc}/lc + \sigma^2{}_e/rlc) \quad (5)$$

where $\sigma^2{}_g$, $\sigma^2{}_e$, $\sigma^2{}_{gc}$, $\sigma^2{}_{gl}$, and $\sigma^2{}_{glc}$ refer to genotypic variance, error variance, genotype × crop cycle interaction, genotype × location interaction, and genotype × location × crop cycle interaction, respectively; *c*, *l*, and *r* represent the crop cycle, locations, and replications.

A hierarchical cluster analysis was conducted on the herbicide phytotoxic percentage and index mean and maximum values for each site using Ward's method and Euclidean distance, as described by Lešková et al. (2017) with R implementation adapted from Hintikka et al. (2022). After clustering, discriminant analysis was performed to evaluate clustering accuracy using DPS software (v. 7.05, Zhejiang University, Hangzhou, China). The discriminant analysis revealed that the maximum values were the most significant metrics for differentiating between clusters based on their high discrimination accuracy rates. The 222 genotypes were classified into five distinct tolerance categories, and an LSD post hoc analysis was conducted for each category using GraphPad Prism software (v. 8, GraphPad Software, Inc., San Diego, CA, USA).

Results and Discussion

Sugarcane-Field Weeds and Control Efficiencies of 81% MAD

Weeds were present at all stages of sugarcane growth and had a significant impact on the crop. During the spring, sugarcane experiences slow growth before canopy closure, leaving fields exposed for prolonged periods (Hajeb et al. 2023). This exposure and high temperatures and humidity promote rapid weed proliferation, impairing sugarcane tillering and seedling development (Mzabri et al. 2022). This leads to substantial reductions in yield and quality, ranging from 30% to 70% under moderate weed infestation, with severe cases resulting in total crop failure (Wen et al. 2021). A comprehensive survey of weed species in sugarcane fields identified 14 species from 8 families in Longzhou, 19 from 12 families in Nongqin, and 19 from 11 families in Qufeng (Table 1). Five weed species were found to be shared across all three locations, including Gramineae: *C. dactylon* and *D. sanguinalis*; Compositae: Oriental false hawksbeard [*Youngia japonica* (L.) DC.]; Convolvulaceae: ivy woodrose [*Merremia hederacea* (Burm. f.) Hallier f.] and white-edge morningglory [*Ipomoea nil* (L.) Roth]; Cyperaceae: *C. rotundus*; and Solanaceae: black nightshade (*Solanum nigrum* L.). A preliminary investigation, supported by a review of relevant literature, identified 116 weed species from

Table 2. Variance analysis for phytotoxic percentage and index.

Source of variation	df	MS	*F*	SS(%)
Phytotoxic percentage				
Genotype (G)	221	12,262	91.61***	44.62
Crop cycle (C)	2	49,003	366.08***	1.61
Location (L)	2	8,821	65.89***	0.29
Replication (R)	2	42,633	318.5***	1.40
L × R	4	2,571	19.21***	0.17
L × year	1	35,672	266.49***	0.59
G × L	442	2,125	15.87***	15.46
G × C	442	2,990	22.34***	21.76
G × L × C	221	2,262	16.9***	8.23
Residuals	2,658	134		5.86
Phytotoxic index				
Genotype (G)	221	9,963	173.9***	45.12
Crop cycle (C)	2	31,150	543.75***	1.28
Location (L)	2	10,286	179.54***	0.42
Replication (R)	2	15,755	275.02***	0.65
L × R	4	1,125	19.63***	0.09
L × year	1	27,933	487.59***	0.57
G × L	442	1,759	30.7***	15.93
G × C	442	2,763	48.22***	25.03
G × L × C	221	1,720	30.03***	7.79
Residuals	2658	57		3.12

***P ≤ 0.001. MS: Mean Square; SS%: Percentage contribution to total sum of squares.

Table 3. Variance analysis on the 2023 phenotypic data for phytotoxic percentage and index.

Source of variation	df	MS	*F*	SS(%)
Phytotoxic percentage				
Genotype (G)	221	7,799	419.739***	59.80
Crop cycle (C)	1	16,983	914.098***	0.59
Location (L)	2	54,799	2,949.416***	1.90
Replication (R)	2	40	2.134	0.00
G × C	221	1,773	95.444***	13.60
G × L	221	3,032	163.199***	23.25
Residuals	1,330	19		0.86
Phytotoxic index				
Genotype (G)	221	6,191	1,236.887 ***	59.76
Crop cycle (C)	1	7,241	1,446.776***	0.32
Location (L)	2	50,795	10,148.514***	2.22
Replication (R)	2	11	2.161	0.00
G × C	221	1,328	265.297***	12.82
G × L	221	2,549	509.198***	24.60
Residuals	1,330	5		0.29

***P ≤ 0.001.
MS, Mean Square; SS%, Percentage contribution to total sum of squares.

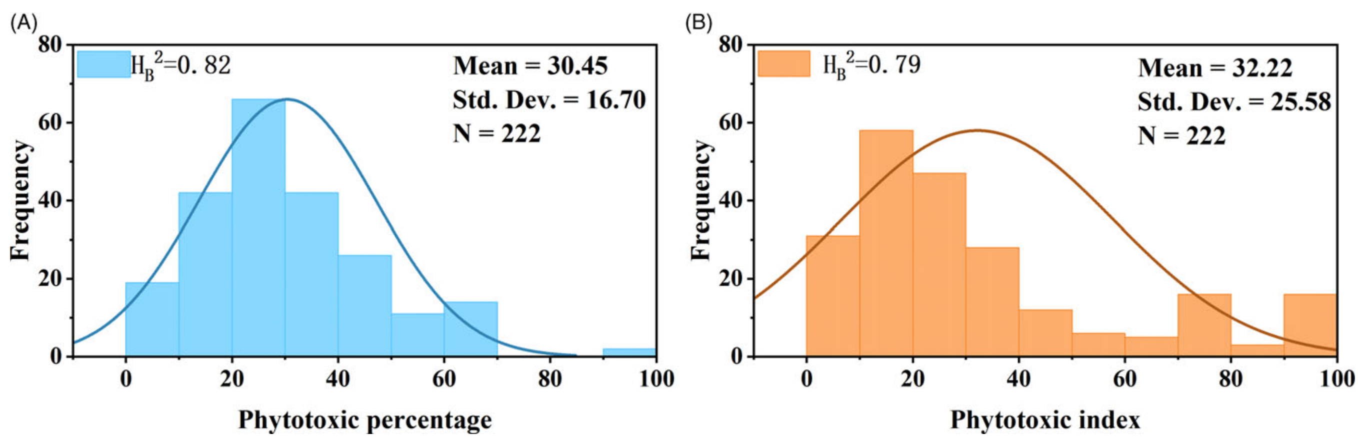

Figure 2. Frequency distribution, heritability of 81% MCPA-ametryn-diuron (MAD) herbicide phytotoxic percentage (*Q*) and herbicide phytotoxic index (PI) of different genotypes across three sites: Longzhou, Nongqin, and Qufeng in Guangxi, China.

27 families as the dominant weed flora in the sugarcane-growing areas of Guangxi (Fu 2008; Lu and Ma 2003; Mayor and Dessaint 1998; Qin and Huang 2014; Xue et al. 2010; Yang 2012). Our study investigated 35 species from 16 families after removing duplicates in three locations. This identification provides a foundation for further weed research in the region and is crucial for selecting the right herbicides and enhancing weed control efficiency in sugarcane fields.

Phenylurea (e.g., diuron), phenoxy (e.g., MCPA-sodium), and triazine (e.g., ametryn) are selective systemic herbicides that absorb through roots, stems, and leaves, disrupting photosynthesis or hormone regulation in meristems to control weeds in crops (Liu et al. 2017). After application of 81% MAD, the control efficiency against *C. rotundus* varied after 7 d, with 26.14% in Longzhou, 36.26% in Nongqin, and 94.93% in Qufeng. However, by day 15, the control efficiency increased significantly, reaching 94.95%, 90.53%, and 96.77% at the respective locations. For *C. dactylon*, the control efficiency was 88.07% after 7 d and reached 100% by day 15, indicating a rapid and effective response to treatment. For *S. nigrum*, control efficiency varied based on plant size; smaller plants responded rapidly. However, the control efficiency across all sites reached 100% within 15 d. Similar responses were observed for *M. hederacea* and *I. nil*, with 90% control in 7 d and 100% by day 15. Most other weed species exhibited control efficiencies exceeding 90% by 15 d posttreatment, except *E. indica*, which demonstrated herbicide tolerance due to its advanced maturity stage. The application of 81% MAD effectively controlled more than 90% of weed species in sugarcane fields within 15 d. However, certain sugarcane varieties might experience growth issues due to sensitivity to these herbicides (Vyver et al. 2013). A 3-yr study conducted in Longzhou, Nongqin, and Qufeng found that herbicide phytotoxicity percentage in sugarcane from May to July varied from 0% to 100%. Symptoms appear 5 to 10 d after application. Mild damage includes dry leaf tips with yellow strips under 5 cm. Severe damage causes leaf chlorosis, desiccation, shrinkage, deformation, stunted growth, and withering of growing points, potentially

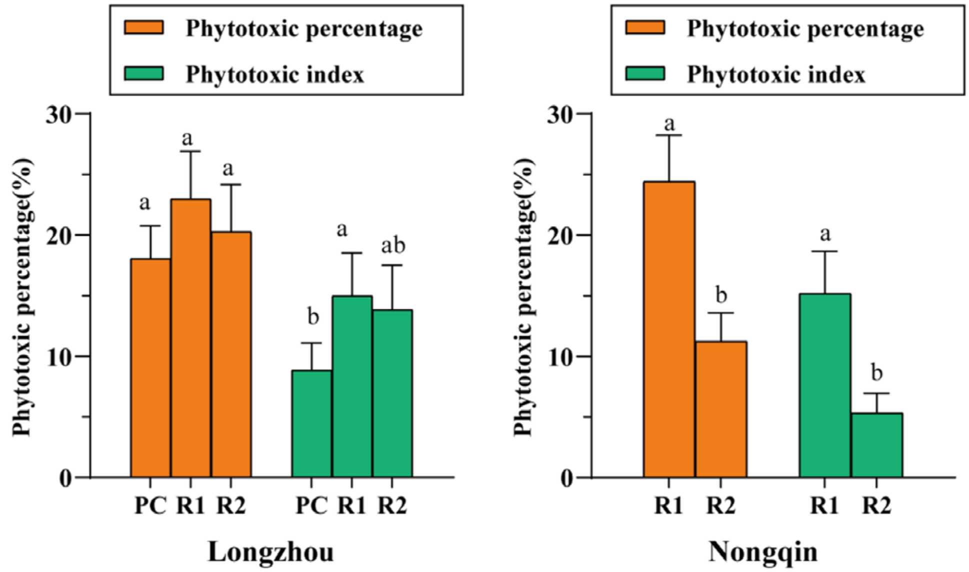

Figure 3. One-way ANOVA for 81% MCPA-ametryn-diuron (MAD) phytotoxic percentage and index for different crop cycles.

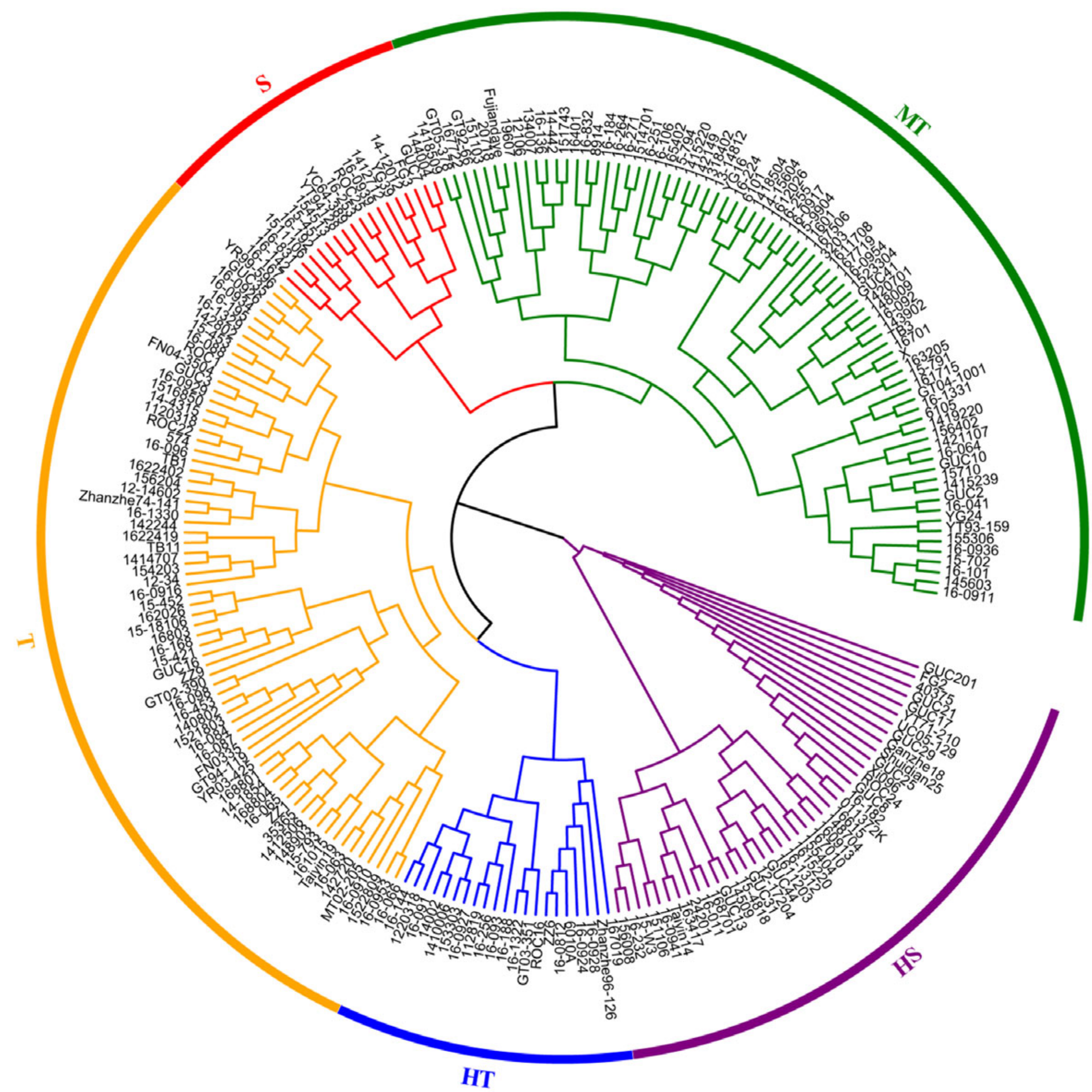

Figure 4. Composite maximum stratified cluster analysis circle plot. HT, highly tolerant; HS, highly susceptible; MT, moderately tolerant; T, tolerant; S, susceptible.

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Table 4. Discriminant accuracy of different clustering metrics.

Clustering basis	Location	Discriminant accuracy %
Average	Longzhou	86.49
	Nongqin	95.50
	Qufeng	95.50
	Combined	93.69
Max	Longzhou	93.69
	Nongqin	95.95
	Qufeng	96.40
	Combined	97.75
Longzhou-Max	Longzhou	93.69
	Nongqin	93.24
	Qufeng	93.24
	Combined	92.79
Nongqin-Max	Longzhou	90.54
	Nongqin	95.95
	Qufeng	98.65
	Combined	92.34
Qufeng-Max	Longzhou	89.19
	Nongqin	90.09
	Qufeng	96.40
	Combined	88.29
Combined-Max	Longzhou	95.05
	Nongqin	98.65
	Qufeng	98.65
	Combined	97.75

leading to the plant's death (Ji 2024; Shan et al. 2020). Each symptom was assessed at varying levels of severity and categorized into a standardized grading system ranging from 0 to 4 across five levels. The phytotoxicity significantly reduces sugarcane yield and sugar content, leading to substantial economic losses (Huang et al. 2021). To minimize such losses, it is crucial to identify herbicide-sensitive cultivars and provide farmers with clear guidance on selecting suitable herbicides for specific sugarcane varieties (Taak et al. 2020). Additionally, the variability in herbicide effects on sugarcane poses a significant challenge in accurately identifying and monitoring herbicide-induced damage, making it a crucial factor in herbicide safety evaluation (Landau et al. 2021). To address these challenges, it is imperative to develop comprehensive technical guidelines for assessing sugarcane tolerance to herbicides and to establish enhanced classification standards for herbicide damage (Wang et al. 2023). This systematic approach provides a reliable method for recording and analyzing herbicide-induced damage in sugarcane. These efforts will contribute to establishing a unified framework for diagnosing and predicting the extent of herbicide damage, ensuring sustainable production and advancement in the sugarcane industry of China (Salgado et al. 2022; Wang et al. 2022).

Variance Analysis of Herbicide Phytotoxicity in Sugarcane

The percentage and index of herbicide phytotoxicity in sugarcane demonstrated a broad distribution across various fields, rendering them highly suitable for tolerance assessment (Figure 2). Combined variance analysis of all collected phenotypic data demonstrated highly significant differences in herbicide phytotoxic percentage and index among genotypes (G, $P < 0.001$), locations (L, $P < 0.001$), and crop cycle (C, $P < 0.001$), as well as their interactions. Such significant interactions were observed for G × L ($P < 0.001$), G × C ($P < 0.001$), and G × L × C ($P < 0.001$). Genotype contributed 44.62% (45.12%) to the variance in herbicide phytotoxicity percentage (index), while location

Table 5. Evaluation criteria and reference genotype for assessing 81% MCPA-ametryn-diuron (MAD) field tolerance in sugarcane.

Tolerance level[a]	Reference variety	Evaluation criterion[b]: Maximum phytotoxic percentage (Q)	Maximum phytotoxic index (PI)
HT	ZZ6, ROC16	$Q \leq 1$ %	PI ≤ 0.5
T	ZZ1, ROC22	1 % < $Q \leq 20$ %	0.5 < PI ≤ 10
MT	GT42, YT93-15	20 % < $Q \leq 50$ %	10 < PI ≤ 25
S	YT94-128, ROC25	50 % < $Q \leq 85$ %	25 < PI ≤ 50
HS	ROC27, YT71-210	$Q > 85$ %	PI > 50

[a]HT, highly tolerant; HS, highly susceptible; MT, moderately tolerant; T, tolerant; S, susceptible.
[b]MAD, MCPA-ametryn-diuron; Q, herbicide phytotoxic percentage (%); PI, herbicide phytotoxic index.

accounted for 0.29% (0.42%) and crop cycle for 1.61% (1.28%). The G × L interaction accounted for 15.46% (15.93%), the G × C interaction for 21.76% (25.03%), and the G × L × C interaction for 8.23% (7.79%) (Table 2). Sugarcane herbicide tolerance was primarily influenced by genotype and crop cycle, although environmental factors also played a significant role.

A variance analysis of phenotypic data collected in 2023 across three locations revealed that genotype (G) ($P < 0.001$), location (L) ($P < 0.001$), crop cycle (C) ($P < 0.001$), the G × L ($P < 0.001$) interaction, and the G × C ($P < 0.001$) interaction significantly influenced herbicide phytotoxic percentage and index. Specifically, genotype accounted for 59.80% of the variance in herbicide phytotoxic percentage, location for 1.90%, crop cycle for 0.59%, G × L for 23.25%, and G× C for 13.60%. Similarly, for the phytotoxic index, genotype accounted for 59.76%, location for 2.22%, crop cycle for 0.32%, G × L for 24.60%, and G× C for 12.82% (Table 3). This suggests that sugarcane tolerance to herbicide phytotoxicity is primarily determined by genotype and crop cycle, while environmental conditions also play a significant role.

ANOVA over the first and the second ratoon cane at the same location indicated significant differences in the ratoon sugarcane in Nongqin. In contrast, no significant differences between the plant cane and the ratoon cane were found in Longzhou (Figure 3). This suggests that sugarcane exhibits varying tolerance levels to herbicide phytotoxicity, with ratoon cane demonstrating greater sensitivity than plant cane. Furthermore, the first ratoon cane shows heightened sensitivity relative to those in the second ratoon cane. Plant cane has a robust root system with high metabolic activity, enabling efficient herbicide phytotoxicity and reduced sensitivity. In contrast, ratoon cane relies on aging roots with diminished nutrient absorption, leading to decreased herbicide tolerance. Its shallow bud points are more exposed to herbicide residues, increasing the risk of damage. The first ratoon cane is weakened by mechanical damage and environmental stress, while the second ratoon cane develops a stronger tolerance over time through root regeneration and soil microorganisms. Plant cane improves stress tolerance through tail fertilizer and moisture-retaining film. In contrast, ratoon cane (especially the first) often suffers from poor management and untimely fertilization, increasing herbicide sensitivity due to malnutrition. Long-term cultivation may trigger adaptive mechanisms, with the second stage potentially reducing herbicide absorption by upregulating tolerance-related genes like ABC transporters (Haj Yasein et al. 2011; Qamar et al. 2021; Thibane et al. 2023).

Root conditions, soil residues, and management practices influence the ratoon cane's sensitivity to herbicides. Future

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Table 6. Variation in the identification indicators of sugarcane tolerance to 81% MCPA-ametryn-diuron (MAD) phytotoxicity among experimental genotypes.

Grade of tolerance[a]	Tested genotype	No.	Multiple comparison	
			Phytotoxic percentage	Phytotoxic index
HT	16-0812, ZZ6, 6010A, 16-0924, 16-0928, GT03-351, Zhanzhe96-126, 167019, 16-1322, ROC16, 16-0927, 153303, 1410006, 14002, 1220318, 16-144, 16-091, 16-093, 16-256, 112819, 16-188	21	3.71 ± 3.35 A	0.93 ± 0.84 A
T	16-066, 16-0953, 1522809, 16-0910, 142720, 16-063, MT02-205, 16-065, 168804, 14-1854, 168801, 148903, 1412506, 35365, ZZ1, 161015, 15-793, Taiyin19, YR03-425, 1521803, 140802, 16-084, 16-087, FN0335, GT94-119, 16-453, 16-098, GT02-390, 15-421, 16-168, GUC16, ZZ9, 16803, 15-18106, 162026, 15-452, 574, ROC22, 16-096, TB1, 1120318, 14-4315, 1516850, 16-0929, GUC3, GUC35, YR99-596, 16-0934, 16-0926, 142802, 16-1329, 16-195, 16-088, 15-453, ROC1, FN04-3504, 156204, 1622402, 12-14602, 142244, 16-1330, Zhanzhe74-141, 154203, 1414707, 12-34, 16-0916, TB11, 1622419	68	18.76 ± 6.05 B	4.69 ± 1.51 B
MT	6105, 1419220, GT04-1001, 16-1331, 161715, YG24, YT93-159, 155306, 16-0936, 15-702, 16-101, 145603, 16-0911, 15710, 1415239, GUC10, GUC2, 16-041, 1421107, 16-064, 156402, 15-9904, 11601, 16-0954, 1523304, 1611708, 167719, 3203, 167506, 16-092, 143902, GUC41, 1420701, 148009, 16701, X, TB3, 163205, 15-791, 14-15604, 1612026, 16-255, 16-0917, 6101, 1418504, YG16, 16-224, 165402, 15-794, 16-106, 16-251, 1415220, 142149, 1318402, 13-14812, 16-832, 8914, 16401, 16-184, 16-271, 1514701, 16-264, 167722, 20718, Fujiandaye, 151103, GT92-66, 1418509, GT05-378, 134007, 16-136, 14-442, 151743, 12106, 19607	75	46.66 ± 7.36 C	23.33 ± 3.68 C
S	154513, YT94128, YC64-389, 16-253, ROC25, 1412712, YG39, 14-12012, 16-0939, GUC7, 144004, FG3, 16-192, 15-451, 1511106, 16-142, 16-831, 16-167	18	71.77 ± 6.54 D	53.83 ± 4.90 D
HS	148704, 160913, 16615, 167010, 155404, GUC31, 15-4818, GUC44, 12-17204, 16-1312, 1611203, 1615220, 24201, 163417, Taiyin14, 168701, 16-011, 14509, GUC13, 16-0941, 151106, 15-W3, 16-232, 156008, 16-182, 011372K, GUC8, ROC24, Xi096, GUC25, Shuidian25, Ganzhe18, GUC29, UC05-129, YT71-210, GUC17, GUC21, 40375, FG2, GUC201	40	94.95 ± 5.46 E	94.95 ± 5.46 E

[a]HT, highly tolerant; HS, highly susceptible; MT, moderately tolerant; T, tolerant; S, susceptible.

research should integrate molecular biology (e.g., tolerance gene screening) with optimized field management (e.g., precise pesticide application) to develop phased herbicide plans. Promoting tolerant varieties like 'Guitang42' and microbial remediation can mitigate pesticide damage and support sustainable production (Cheeke et al. 2012).

Tolerance Evaluation by Cluster and Discriminant Analysis

Hierarchical cluster analysis constructs a hierarchy of clusters by evaluating the similarity or distance between data samples (Gupta et al. 2022; Kalogiouri et al. 2021). The maximum and average phytotoxic percentages and indices were calculated independently and in combination for each experimental site. Hierarchical cluster analysis classified 222 sugarcane genotypes into five categories, each undergoing individual self-discriminant analysis (Figure 4). The accuracy of cluster analysis based on the maximum values was significantly higher than that based on the average values, demonstrating that using maximum values resulted in more accurate and stable classifications. Therefore, the maximum values were utilized in subsequent analyses.

The discriminant analysis classifies groups based on eigenvalues under categorical conditions (Ramsey et al. 2012). The interaction discrimination among the three experimental sites and the combined clustering analysis results revealed that the combined maximum values achieved an accuracy of greater than 95%. The discriminant analysis accuracy was 95.05% for Longzhou, 98.65% for Nongqin, and 98.65% for Qufeng (Table 4). This indicates that cluster analysis using combined maximum values provides a more accurate and widely applicable classification. Combining these methods offers a robust approach for accurately categorizing experimental genotypes (Xu et al. 2023).

Initially, mean and maximum values were used in clusters and discriminant analyses. However, the maximum values yielded higher discriminant accuracy, indicating that maximum incidence is a more reliable measure of varietal tolerance (Jiang et al. 2024). To accurately assess herbicide phytotoxic tolerance in sugarcane, widely cultivated genotypes representing different tolerance levels were selected as reference controls (Table 5). Over 3 yr of trials conducted in Longzhou, Nongqin, and Qufeng, ROC22 consistently demonstrated tolerance, with a herbicide phytotoxic percentage below 20%, establishing it as a reliable tolerant control. Conversely, highly susceptible genotypes such as YT94-128, ROC25, YT71-210, and ROC27 exhibited maximum phytotoxic percentages ranging from 50% (susceptible) to greater than 85% (highly susceptible). These results confirm the utility of these genotypes as susceptible controls due to their consistently high damage levels across all tested regions. This study applied self-discriminatory and interactive discriminatory analyses to evaluate individual locations and perform comprehensive cross-site assessments. A total of 222 sugarcane genotypes were classified into five distinct categories using clustering and discriminant analysis based on the combined maximum value approach. The classifications are as follows: 21 highly tolerant (HT) genotypes (9.5%), 68 tolerant (T) genotypes (30.6%), 75 moderately tolerant (MT) genotypes (33.8%), 18 susceptible (S) genotypes (8.1%), and 40 highly susceptible (HS) genotypes (18%) (Table 6; Supplementary Table S1). The classifications derived from individual locations were validated across other experimental sites, establishing widely applicable classification criteria (Li et al. 2024). The significant contribution of the G × L interaction underscores the complexity of tolerance mechanisms. These findings emphasize the vital role of parental genotype selection in breeding programs aimed at developing sugarcane cultivars with improved herbicide tolerance. This might also explain the challenges associated with breeding for herbicide tolerance.

Evaluation of Sugarcane Parents for Tolerance to 81% MAD

Parental traits significantly influence progeny tolerance (Xu et al. 2025). In our study, most parents exhibited pedigrees of tolerant or

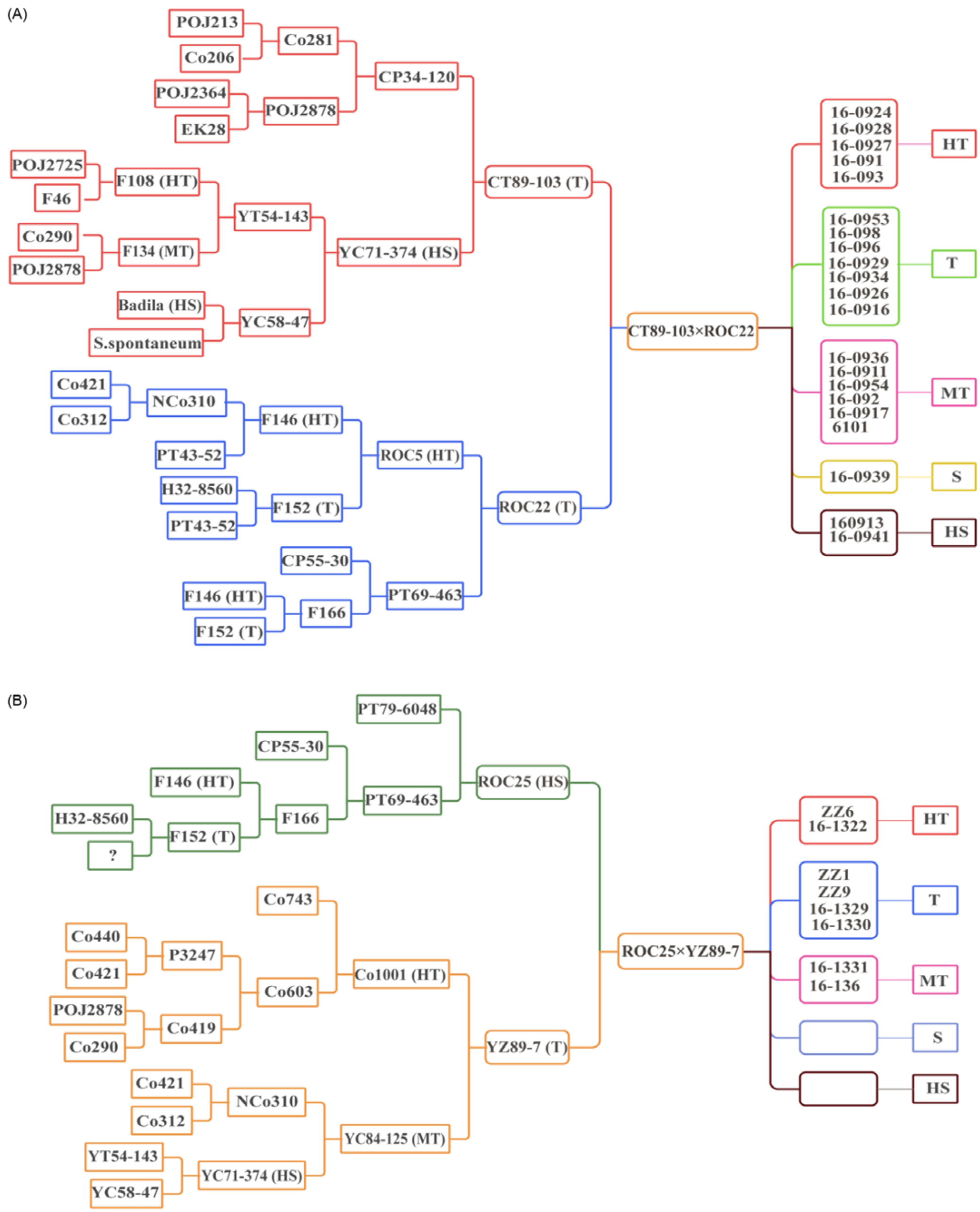

Figure 5. Parent traceability analysis of four important sugarcane varieties. (A) Progeny tolerance distribution of cross CT89-103 × ROC22; (B) Progeny tolerance distribution of cross ROC25 × YZ89-7. A question mark (?) indicates no parental information available. HT, highly tolerant; HS, highly susceptible; MT, moderately tolerant; T, tolerant; S, susceptible.

highly tolerant sugarcane varieties, including CT89-103, ROC22, and YZ89-7. The progenies of CT89-103 × ROC22 comprised 21 genotypes, with 18 exhibiting moderate to high tolerance (Figure 5A; Supplementary Table S2). Moreover, other parents crossed with ROC22, such as Co1001 (HT), YT93-124 (MT), and GT92-66 (MT), exhibited high or moderate tolerance to the herbicide. Among their 18 offspring genotypes, 16 exhibited moderate to high tolerance, including GT04-1001 (MT), GT05-378 (MT), 14-2802 (T), and 16-0812 (HT), while only two exhibited susceptibilities (Supplementary Table S2). This suggests that using tolerant parents increases the likelihood of obtaining tolerant progeny. The progeny of ROC25 × YZ89-7 were also examined, revealing that eight of the genotypes evaluated in this study exhibited moderate or high tolerance. Conversely, zero genotypes exhibited susceptibility (Figure 5B). The inclusion of tolerant parents appeared more favorable for obtaining tolerant progeny. Lineage analyses of ROC22, CT89-103, ROC25, and YZ89-7 indicated these varieties are closely related, with their lineages, YC71-374, F146, and F152, being utilized multiple times (Figure 5). When both parents are tolerant, the majority of progeny inherit tolerance. A substantial proportion of progeny may still exhibit tolerance if one parent is susceptible. However, when both parents are susceptible, the likelihood of producing tolerant progeny decreases significantly (Brahimi et al. 2020). Lineage analysis revealed that ROC22, CT89-103, ROC25, and YZ89-7 are closely related, with lineages YC71-374, F146, and F152 frequently utilized. Herbicide-tolerant sugarcane progenitors are pivotal in breeding herbicide-tolerant varieties.

Conventional breeding is the most commonly used method in crop genetic breeding, and the effectiveness of a parent depends on its ability to select and breed suitable progeny varieties (Li et al. 2018). The current study identified 89 tolerant genotypes, including ROC22 and its hybrid progenies 16-1715 and 16-041, displaying a maximum herbicide phytotoxic percentage and index of less than 20% and 10%, respectively. Conversely, 58 susceptible varieties, such as ROC25 and YT71-210, exhibited maximum phytotoxic percentage and index exceeding 50% and 85%, respectively. Notably, only 9.5% of the genotypes were classified as HT, indicating the urgent need to develop more herbicide-tolerant cultivars. Future breeding efforts should enhance these progenitors' genetic diversity and prioritize using herbicide-tolerant parents to facilitate genetic improvement.

This study identified valuable genetic material among sugarcane parental lines and hybrid progenies, providing critical resources for breeding programs to develop herbicide-tolerant varieties and expand the pool of HT genotypes. A total of 21 HT genotypes were identified as strong candidates for use as parents in breeding programs aimed at enhancing tolerance to herbicide damage. By strategically integrating cluster and discriminant analyses, this study provides a robust framework for evaluating herbicide tolerance across diverse experimental sites, which has the potential for broader applications in assessing various forms of herbicide damage. Future research should prioritize evaluating novel germplasm, identifying tolerance-related genes, and optimizing breeding strategies to accelerate the development of sugarcane varieties with improved herbicide tolerance. This systematic approach enhances our understanding of tolerance mechanisms and paves the way for improving sugarcane tolerance, productivity, and sustainability in the face of herbicide challenges.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2025.10033

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