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Screening, dose-response, and management of Echinochloa crus-galli biotypes to bispyribac-sodium: Implications for histophysiological characteristics, agronomic attributes, and amylose in irrigated rice

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Screening, dose-response, and management of *Echinochloa crus-galli* biotypes to bispyribac-sodium: Implications for histophysiological characteristics, agronomic attributes, and amylose in irrigated rice

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ABSTRACT

The presence of barnyardgrass negatively affects productivity in irrigated rice. Although farmers predominantly used the bispyribac-sodium herbicide, prolonged application has inadvertently led to resistance, making it necessary to assess resistance levels for guided control strategies that combine herbicides with different modes of action (MoA). Therefore, this study aimed to (1) determine the resistance index of barnyardgrass to bispyribac-sodium herbicide, (2) identify active ingredients (a.i.) in mixed herbicides for controlling resistant barnyardgrass biotype and to evaluate the effects on histophysiological, agronomic attributes, and amylose content in irrigated rice. The experiment started by collecting barnyardgrass seeds from five accessions in irrigated rice fields, and then sprayed with the bispyribac-sodium at the recommended dose (103 g a.i.ha^{-1}) from April to July 2023. The seeds in the resistant category were continued in dose-response testing at 0, 1/16, 1/8, 1/4, 1/2, 1, 2, 4, 8, and 16-fold the recommended dose from August to November 2023. Biotype progenies from low, labeled, and high-doses were further evaluated for resistance management using cyhalofop-butyl+penoxsulam, florpyrauxifen-benzyl+cyhalofop-butyl, fenoxaprop-P-ethyl+ethoxysulfuron, and bentazone+MCPA at the recommended dose from July to December 2024. Parameters were observed and analyzed by ANOVA and DMRT. The results showed that

the SB3-accession was 9.82-fold more resistant to the bispyribac-sodium herbicide compared to the susceptible. The fenoxaprop-P-ethyl+ethoxysulfuron demonstrated 100% effectiveness in controlling barnyardgrass of resistant biotype progenies, thereby increasing irrigated rice productivity and amylose content were 27.01 and 31.50%. In conclusion, this study achieved effective management of resistant-biotype by combination of herbicides with different MoA.
Keywords: barnyardgrass; bispyribac-sodium; herbicide mixture; identification; resistance.

Abbreviations

MoA	Modes of action
a.i.	Active ingredients
P_n	Photosynthesis rate
E	Transpiration rate
C_i	Intercellular CO_2 concentration
g_s	Stomatal conductance
SB3	Barnyardgrass accession from Sei Bamban Subdistrict
MDN	Barnyardgrass accession from Medan City
MCPA	2-methyl-4-chloro-phenoxyacetic acid
ALS	Acetolactate synthase
ACCase	Acetyl-CoA carboxylase
R	Resistant
DR	Developing resistance
S	Susceptible
GR_{50}	Growth reduction to decrease the weed growth by 50%
P1	SB3 biotype progeny at low-dose (1/16-fold)
P2	SB3 biotype progeny at labeled dose (1-fold)
P3	SB3 biotype progeny at high-dose (8-fold)
M0	Unsprayed
M1	Cyhalofop-butyl+penoxsulam
M2	Florpyrauxifen-benzyl+cyhalofop-butyl
M3	Fenoxaprop-P-ethyl+ethoxysulfuron
M4	Bentazone+MCPA

INTRODUCTION

Barnyardgrass (*Echinochloa crus-galli* L.) is among the principal weeds in irrigated rice fields. These weeds pose a serious challenge to crop production due to the rapid growth, reaching flowering within 67-76 days (Chen et al. 2022), abundant seed production ranging from 1,320-1,579 seeds per plant (Awan and Chauhan 2016), and aggressive competition for macronutrient uptake and photosynthesis rate (Zeng et al. 2011; Awan et al. 2024). The competition significantly reduced the irrigated rice productivity by up to 58% at a density of 8 populations per square meter (Wang et al. 2019) and even led to losses of up to 83.59% at 20 populations per irrigated rice plant (Tampubolon et al. 2025). To achieve optimal irrigated rice

production, these weeds should be controlled during the critical period between 15 and 45 days after planting (Ankit et al. 2018). However, farmers control efforts are largely dependent on the use of herbicides. Based on interviews with farmers in irrigated rice production centers in Indonesia, particularly in Serdang Bedagai District, North Sumatra Province, current control practices rely significantly on chemical herbicides. Active ingredients commonly applied in this location include single herbicides, such as bispyribac-sodium, 2,4-D dimethylamine, fenoxaprop-P-ethyl, and profoxydim, as well as mixed herbicides, namely cyhalofop-butyl+ethoxysulfuron, metsulfuron-methyl+chlorimuron-ethyl+2,4-D sodium salt, bensulfuron-methyl+bispyribac-sodium, and bispyribac-sodium+pyrazosulfuron. Farmers selected this herbicide for its effectiveness in controlling these weeds during the post-emergence phase. However, the increased use of herbicides with a similar mode of action (MoA), particularly acetolactate synthase (ALS) inhibitors such as bispyribac-sodium, has created a new problem, namely resistance. Continuous, long-term application of herbicides with a similar MoA creates strong selection pressure, favoring the development of resistant biotype which are difficult to control. Ineffective control of barnyardgrass poses a persistent challenge for farmers, as evidenced in Sei Bamban, Serdang Bedagai District, North Sumatra, Indonesia.

This problem underscores the need to identify and classify the resistance of barnyardgrass to the bispyribac-sodium herbicide through screening and dose-response testing. Presently, there have been no reports of barnyardgrass resistance to bispyribac-sodium in the study area. The first case of bispyribac-sodium resistant barnyardgrass in Indonesia was reported by Rasyid et al. (2022), who found that the barnyardgrass population in irrigated rice fields in South Sulawesi had shown low resistance (5.61-fold). Screening and dose-response testing have also demonstrated that weeds are resistant to the herbicide (Tampubolon et al. 2019; Marchesi and Saldain 2019; Gobi et al. 2025). Fuentes et al. (2025) reported that barnyardgrass in irrigated rice fields from Ecuador demonstrated 16.1-fold resistance to bispyribac-sodium compared to susceptible populations. This assessment is important as an initial step to determine resistance classification, typically expressed as a resistance index based on the growth reduction ratio of resistant to susceptible. By understanding the level of resistance, farmers and agronomists can inform decisions regarding effective management strategies for resistant biotypes using alternative herbicides. According to Monaco et al. (2002), herbicide-resistant weed management can be achieved by alternating MoA herbicides, rotating MoA herbicides during crop rotation, and mixing herbicides with different MoA. Resistance management using herbicide mixtures with different MoA and rotation has been reported to be effective in suppressing resistant-biotype (Zhang et al. 2023; Tampubolon et al. 2024). Gobi et

al. (2025) reported that the 40:60 mixture of propanil and quinclorac was highly effective in suppressing resistant barnyardgrass biotype to bispyribac-sodium with a mortality rate up to 95%. Zia-Ul-Haq et al. (2019) also found that the combination of fenoxaprop-P-ethyl and ethoxysulfuron herbicide was highly effective in controlling barnyardgrass ranging from 84 to 94%. Therefore, the application of rotation strategies and combinations of herbicides with different MoA is the key to managing resistant-biotype.

To obtain comprehensive results and provide useful solutions, screening and dose-response testing should be combined with the management of resistant-biotype by mixing herbicides with different MoA. However, the application of this herbicide mixture might have a negative or positive impact on the amylose content of irrigated rice grains. The amylose content is a major determinant of various quality attributes of cooked rice, including taste, texture, and appearance. The amylose content of rice grains ranging from 16-24%, is preferred for general consumption due to the balanced texture and pleasant taste (Li et al. 2024). According to Akbar et al. (2011), the use of butachlor, pendimethalin, and pretilachlor herbicides at a dose of 1.8, 1.65, and 1.25 kg ha^{-1} significantly inhibited the amylose content of irrigated rice grains by 3.26, 8.75, and 5.98% compared to the control. Other studies showed the inverse, as the mixed herbicides of pendimethalin+ethoxysulfuron, pyrazosulfuron+ethoxysulfuron, bispyribac-sodium+ethoxysulfuron, and penoxsulam+ethoxysulfuron significantly increased amylose content in rice grains compared to the control (Khaliq et al. 2012). These contrasting results indicate that the effect of herbicides on amylose content is highly specific to active ingredients. Therefore, it is important to further examine the effects of mixed herbicides to ensure that effective weed control strategies do not compromise the quality of irrigated rice.

Based on the issues faced by farmers regarding the failure to control barnyardgrass in irrigated rice fields, comprehensive solutions involving screening tests, dose-response, and the management of resistant-biotypes by the mixing of herbicides with different MoA can be implemented. Furthermore, efforts to control these resistant biotypes are intended not only to secure yields quantitatively but also qualitatively. Therefore, this study aimed to (1) identify and determine the resistance index of barnyardgrass to bispyribac-sodium herbicide in irrigated rice fields, and (2) identify active ingredients of mixed herbicides effective in controlling bispyribac-sodium resistant barnyardgrass and evaluate the effects on the histophysiological characteristics, agronomic attributes, and amylose content of irrigated rice grains. The results are expected to be useful to farmers and agronomists in making informed decisions on the management of herbicide-resistant barnyardgrass.

MATERIALS AND METHODS

Experiment 1. Screening Testing

During screening, barnyardgrass seeds were collected from irrigated rice fields by Alridiwirsah et al. (2023a) across five accessions in North Sumatra, Indonesia: SB1-SB5 (Sei Bamban Subdistrict, Serdang Bedagai District) and MDN (Padang Bulan, Medan City). The SB accession was selected based on a six-year history (2018-2023) of intensive exposure to the bispyribac-sodium herbicide used by farmers, either singly or in combination with other active ingredients. The MDN accession was selected as the comparison for manual weed control (Figure 1 and Supplementary 1). Farmers predominantly used bispyribac-sodium solely with an intensity of 29.49%, and some also applied herbicide combinations containing a similar active ingredient, such as bensulfuron+bispyribac-sodium (12.18%) and bispyribac-sodium+pyrazosulfuron (8.33%). The consistent use of herbicides with a similar active ingredient by farmers makes barnyardgrass difficult to control, suggesting the development of resistance.

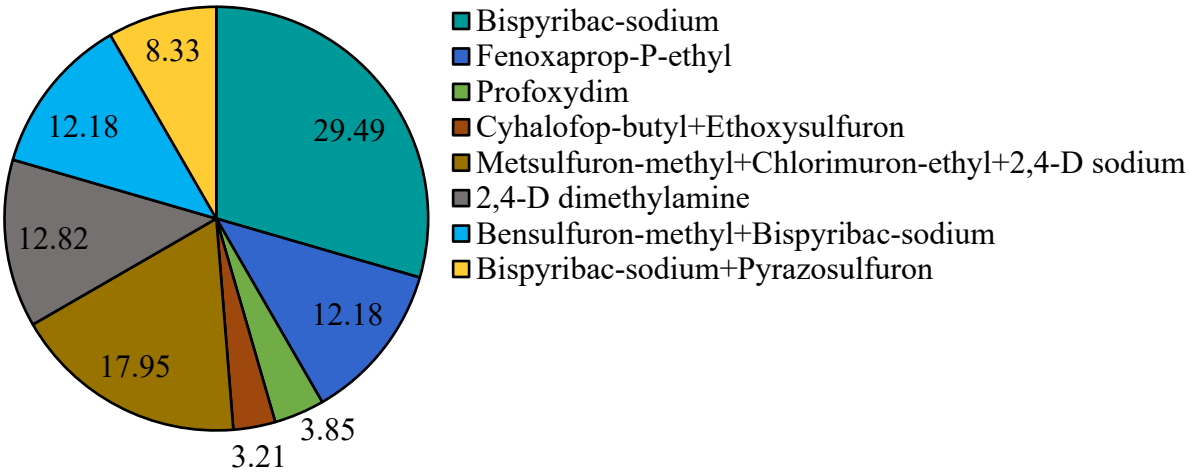


Figure 1. Intensity of herbicide active ingredient application by irrigated rice farmers for weed control across growing seasons from 2018 to 2023.

Barnyardgrass seeds of SB1-SB5 and MDN were germinated in rice field soil in buckets with an upper diameter of 37.5 cm, a height of 15 cm, and a lower diameter of 28 cm. At the 2-3 leaf stage, 20 seedlings were transplanted into each bucket. This screening study was conducted at Padang Bulan, Medan Selayang, North Sumatra, Indonesia (03°33'40.14"N; 98°38'51"E) from April to July 2023, following a non-factorial Randomized Complete Block Design (RCBD) with four replications. At the 4-leaf stage, the recommended dose of bispyribac-sodium herbicide was sprayed at 103 g a.i.ha^{-1} (Nominee 103 OF). Spray calibration was performed, yielding a result of 332.28 L ha^{-1}. The parameters of survival rate and number

of tillers at three weeks after application (WAA) were measured. Subsequently, resistance was classified as susceptible (S) when the survival percentage was less than 2%, developing resistance (DR) ranged from 2 to 19%, and resistant (R) was equal to or greater than 20% (Owen and Powles 2009). At 9 WAA, the number of seeds was manually determined, and shoots were oven-dried at 65°C for 72 h to obtain dry weight. Data were processed using one-way ANOVA followed by DMRT at $P<0.05$. One accession with the highest survival rate was selected, and the seeds were air-dried for dose-response testing.

Experiment 2. Dose-Response Testing

In the dose-response testing, the SB3 biotype seeds with the highest survival rate and the MDN population served as a comparison were used. The study site and procedures from germination to transplanting both the SB3 biotype and MDN were performed in accordance with the screening protocol. After growing four leaves, bispyribac-sodium herbicide was applied at the specified rate. This study was conducted from August to November 2023 using a factorial RCBD with three replications. The first factor was the SB3 biotype (resistant) and the MDN (susceptible), while the second factor was the bispyribac-sodium herbicide (0, 1/16, 1/8, 1/4, 1/2, 1, 2, 4, 8, and 16-fold the recommendation dose or equivalent to 0; 6.44; 12.88; 25.75; 51.5; 103; 206; 412; 824; and 1,648 g a.i. ha^{-1}). Spray calibration was performed, yielding a result of 293.89 L ha^{-1}. Parameters in this testing included survival percentage measured at 3 WAA, SPAD total chlorophyll at 7 days after application (DAA) using a SPAD-502Plus chlorophyll meter, manually calculated seed numbers, and dry weight at 8 WAA by oven-drying at 65°C for 72 h. Furthermore, growth reduction (GR_{50}) was calculated from the dry weight of the resistant biotype and susceptible using log-logistic regression, and the resistance index was derived from the GR_{50} ratio of resistant and susceptible. Data were analyzed using ANOVA followed by DMRT at $P<0.05$ in Excel, and GR_{50} was calculated in IBM SPSS software. Seeds of the barnyardgrass resistant-biotype (SB3) from each low, labeled, and high-dose were harvested and air-dried for resistance management.

Experiment 3. Management of Resistant-biotype Progenies by Mixed Herbicides

Germination

The rice field soil was taken from Sampora Village, Cibinong (06°30'3.6"S; 106°50'58.3"E), while the study was conducted from July to December 2024 on farmers land in Tarikolot Village (06°30'8.16"S; 106°49'30.4"E), Nanggewer Mekar, Cibinong Subdistrict, Bogor District, West Java, Indonesia, at an elevation of 160 m above sea level. The procedure started with the collection of rice field soil, which was placed in buckets measuring 33 cm in diameter and 12 cm high. Seeds of the resistant-biotype progeny (SB3) at low (1/16-fold),

labeled (1-fold), and high-dose (8-fold) or 6.44; 103; and 824 g a.i.ha^{-1} were taken from a dose-response testing. Meanwhile, the irrigated rice variety used was Inpari-32. The irrigated rice and barnyardgrass biotype seeds were germinated until 3-4 leaves appeared, followed by transplantation. The irrigated rice seedlings were planted in the center and the barnyardgrass biotypes were planted in a circle at a distance of 5 cm from the plant stem using a 1:5 ratio.

Study Design and Spraying Calibration

This study used a factorial RCBD with the first factor being the resistant-biotype progenies of barnyardgrass (P1= SB3 at low-dose or 1/16-fold, P2= SB3 at labeled-dose or 1-fold, and P3= SB3 at high-dose or 8-fold). The second factor was mixed herbicides, namely M0= unsprayed, M1= cyhalofop-butyl 50 g L^{-1} + penoxsulam 10 g L^{-1} (Topshot 60 OD, Syngenta), M2= florpyrauxifen-benzyl 20 g L^{-1} + cyhalofop-butyl 100 g L^{-1} (Novlect 120 EC, Corteva), M3= fenoxaprop-P-ethyl 69 g L^{-1} + ethoxysulfuron 20 g L^{-1} (Ricestar Xtra 89 OD, Bayer), and M4= bentazone 400 g L^{-1} + MCPA (2-methyl-4-chloro-phenoxyacetic acid) 60 g L^{-1} (Basagran 460 SL, BASF) with a recommended dose of 1 L ha^{-1}. Each treatment combination was replicated three replicates and buckets were arranged at a spacing of 25 cm × 50 cm. Spray calibration was performed, yielding a result of 345.74 L ha^{-1}.

Cultivation Practices and Herbicide Application

Basic fertilization was carried out using a compound NPK fertilizer at a dose of 200 kg ha^{-1} (equivalent to 2.5 g per bucket). The fertilizer was applied as two equal doses of 1.25 g per bucket, the first three days before transplanting and the second at the end of the vegetative period of irrigated rice plants. The fertilizer was dissolved in 100 mL of water and then evenly applied to the soil surface. Data on the growth of irrigated rice and barnyardgrass biotype were recorded before spraying. After the barnyardgrass resistant-biotype had 5-6 leaves, the mixed herbicides were sprayed according to the treatment. Spraying was carried out at 10:30 AM Indonesia time with a temperature, humidity, and air pressure of 31.7°C, 55%, and 1,011 hPa, respectively. Pest and disease control in irrigated rice plants was carried out using deltamethrin and mancozeb according to the prevailing incidence of infestation.

Data Collection and Analysis

This study collected data on barnyardgrass resistant-biotype progenies and irrigated rice (Table 1). Physiological, histological, and leaf characteristic measurements (length, width, and area) were performed on the first fully opened leaf of the barnyardgrass resistant-biotype shoot, while the second leaf was measured on the irrigated rice plants. Physiological measurements were conducted from 8:30 to 11:00 AM Indonesia time, and flowering conditions were observed daily. After measuring panicle length, observations were made to confirm that the

seeds were physiologically mature. Barnyardgrass resistant-biotype seeds were harvested every two days based on the criterion of shedding seeds upon handling. The number of seeds from each harvest was counted and tabulated at the end of the generative period. This differed from the criterion for irrigated rice grains, characterized by yellowing of more than 80%. Harvest index, productivity, and yield increase over control (YOC) of irrigated rice were calculated using equations 1-3 based on Bharati et al. (2000); Anwar et al. (2012), while weed control efficiency (WCE) was calculated using equation 4 according to Hasanuzzaman et al. (2008).

$$\text{Harvest index} = \frac{\text{Weight of filled grains}}{\text{Weight of filled grains + Dry weight}} \times 100\% \quad (1)$$

$$\text{Productivity} = \frac{\text{Weight of filled grains (g)}}{1{,}000{,}000} \times \frac{10{,}000\ \text{m}^2}{\text{Planting distance area } (0.125\ \text{m}^2)} \quad (2)$$

$$\text{YOC} = \frac{\text{Rice productivity in herbicide} - \text{Rice productivity in untreated}}{\text{Rice productivity in untreated}} \times 100\% \quad (3)$$

$$\text{WCE} = \frac{\text{Weed dry weight in untreated} - \text{Weed dry weight in herbicide}}{\text{Weed dry weight in untreated}} \times 100\% \quad (4)$$

Irrigated rice grains were husked, weighed 10 g, ground, and sieved through an 80-mesh sieve to produce rice flour. Amylose content was determined by weighing 100 mg of rice flour into a 100 mL volumetric flask. Subsequently, 1 mL of 95% ethanol and 9 mL of 1 N NaOH were added, respectively. The mixture solution was left overnight, and the solution was diluted to 100 mL with distilled water. A 5 mL solution was pipetted into a 100 mL volumetric flask, followed by the addition of 1 mL 0.5 N acetic acid and 2 mL of iodine solution. The solution was further diluted to 100 mL with distilled water, and the absorbance was measured. The standard solution was prepared by weighing 100 mg of potato amylose, then adding 1 mL of 95% ethanol and 9 mL of 1 N NaOH. The solution was heated in a water bath for 10 minutes, cooled for 1 hour, and diluted to a final volume of 100 mL with distilled water. The amylose standard solution was pipetted at 0.2; 0.4; 0.6; 0.8; and 1 mL, then 2 mL of iodine solution and 1 mL of 0.5 N acetic acid were added to each concentration. The standard solution was diluted to 100 mL with distilled water, vortexed, left for 20 minutes, and the absorbance was measured using a UV-vis spectrophotometer at a 620 nm wavelength. Amylose content was expressed as a percentage and classified as very low (less than 10%), low (10-19.99%), moderate (20-25%), or high (greater than 25%) according to Kumar and Kush (1986). Parameters were analyzed using ANOVA and followed by DMRT at $P<0.05$ using IBM SPSS software. Additionally, a Pearson correlation analysis was performed to assess the relationships between histophysiological characteristics and yield attributes of irrigated rice.

319 Table 1. Parameters and measurement instruments of barnyardgrass resistant-biotypes and irrigated rice in this study.

Characteristics	Barnyardgrass resistant-biotypes	Irrigated rice	Instruments	Measurement Time
Vegetative	Weed height	Plant height	Measuring tape	21 DAA
	Number of tillers	Number of tillers	Observation	
	Leaf length (l)	Leaf area= l×w×c, constant	Digital caliper	
	Leaf width (w)	(c)=0.76 (Gomez and Gomez 1976).		
Physiological	SPAD total chlorophyll	SPAD total chlorophyll	SPAD-502Plus chlorophyll meter	1, 3, 5, 7, 21 DAA
	Photosynthesis rate	Photosynthesis rate	LI-6800 portable photosynthesis system	7 DAA
	Transpiration rate	Transpiration rate		
	Intercellular CO_2 concentration	Intercellular CO_2 concentration		
	Stomatal conductance	Stomatal conductance		
Histological	Upper epidermis	Upper epidermis	Paraffin method (Johansen 1940)	7 DAA
	Mesophyll	Mesophyll		
	Lower epidermis	Lower epidermis		
	Stomatal density	Stomatal density	Microscopic	
Yield attributes	Flowering age	Flowering age	Observation	Early generative phase
	Panicle length	Panicle length	Digital caliper	Late generative phase
	Number of seeds	Number of filled grains	Observation	
	Dry weight (65°C, 72 h)	Dry weight (80°C, 48 h)	Oven-drying and analytical balances	
	Flag leaf length and width	Flag leaf area	Digital caliper	
		Number of productive tillers	Observation	
		Weight of filled grains	Analytical balances	
		1000-grains weight	Analytical balances	
		Harvest index	Conversion	
		Productivity (ton ha^{-1})	Conversion	
Quality		Amylose content	UV-vis spectrophotometer at 620 nm	Post-harvest

320 Note: DAA= days after application

RESULTS

Experiment 1. Screening Testing

The results showed that the application of bispyribac-sodium herbicide at 103 g a.i.ha^{-1} had an insignificant effect on survival rate, number of tillers and seeds, as well as dry weight of several barnyardgrass accessions (Figure 2). The SB3 accession was confirmed to be resistant, while SB1, SB2, SB4, and SB5 accessions were classified as developing resistance. The MDN population was susceptible to bispyribac-sodium. Additionally, the SB3 accession produced a higher number of tillers and seeds, as well as dry weight compared to other SB accessions.

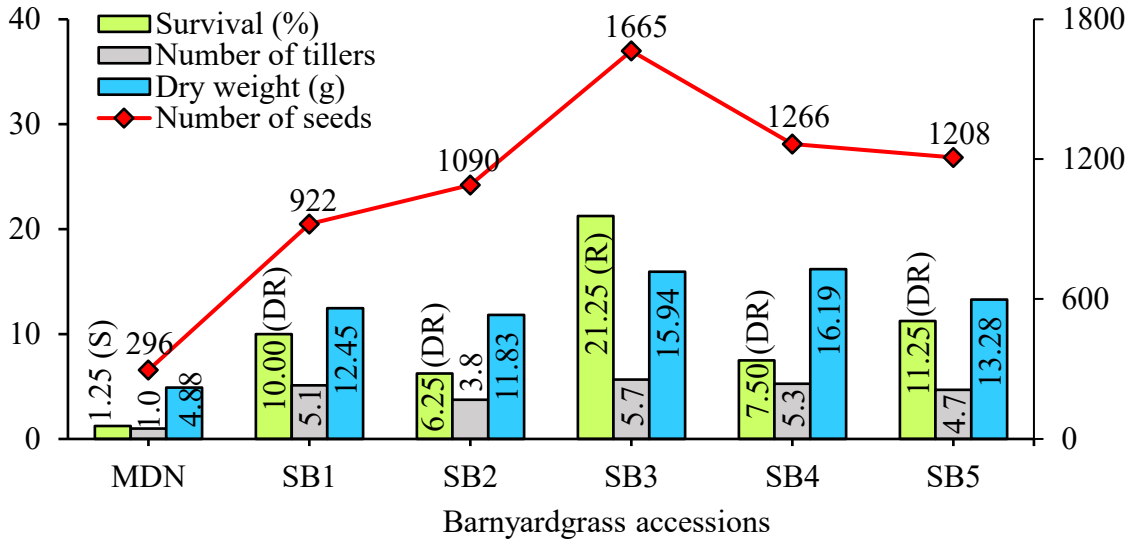


Figure 2. Screening of barnyardgrass accessions from Medan (MDN) and Sei Bamban (SB) for resistance to bispyribac-sodium herbicide. Resistance classification based on survival rate into three groups: susceptible (S <2%), developing resistance (DR 2-19%), and resistant (R ≥20%).

Experiment 2. Dose-Response Testing

ANOVA showed that biotypes and bispyribac-sodium doses significantly affected the survival percentage, SPAD total chlorophyll, number of seeds, and dry weight of barnyardgrass (Table 2 and Figure 3). Similarly, the interaction between biotypes and herbicide doses significantly affected survival and dry weight. As shown in Figure 2, both the SB3 biotype and the MDN population progressively inhibited survival, SPAD total chlorophyll, number of seeds, and dry weight of barnyardgrass as the herbicide dose increased to 16-fold the recommended. Bispyribac-sodium at 16-fold the recommended dose effectively controlled 100% of the barnyardgrass resistant-biotype (SB3). Additionally, the resistance index indicated that the SB3 biotype demonstrated 9.82-fold more resistance compared to the susceptible population (Table 3).

346 Table 2. Analysis of variance for the barnyardgrass growth of resistant-biotype (SB3) and
347 susceptible (MDN) under dose-response to bispyribac-sodium herbicide.

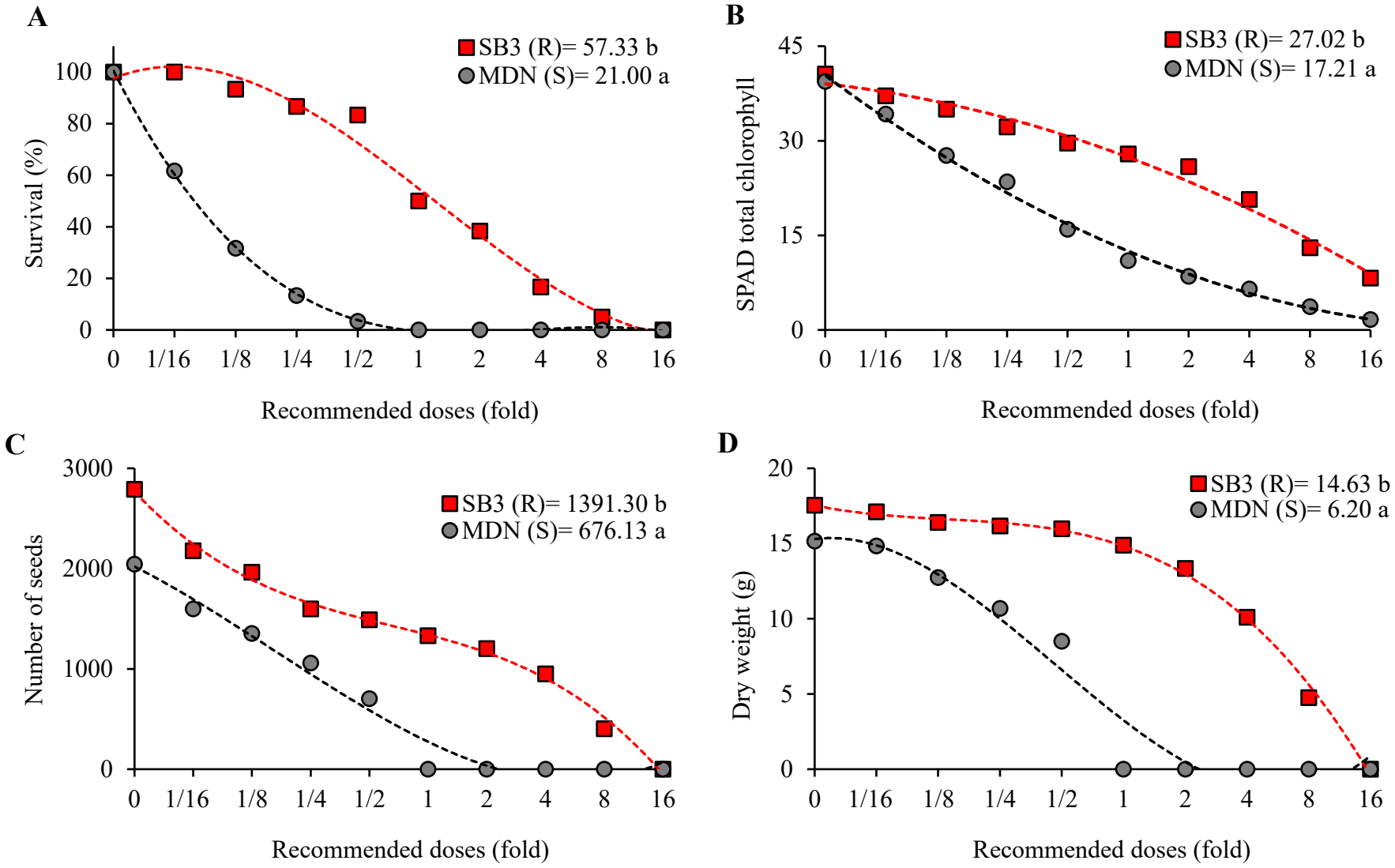
Parameters	Source of variation	df	Sum of squares	Mean square	F-value	$F_{5\%}$	$F_{1\%}$
Survival (%)	Replicates	2	15.83	7.92	0.16^{ns}	3.25	5.21
	Treatments	19	95258.33	5013.60	103.87^{**}	1.87	2.43
	Barnyardgrass (B)	1	19801.67	19801.67	410.25^{**}	4.10	7.35
	Doses (D)	9	63275.00	7030.56	145.66^{**}	2.14	2.91
	B×D	9	12181.67	1353.52	28.04^{**}	2.14	2.91
	Error	38	1834.17	48.27			
	Total	59	97108.33				
SPAD total chlorophyll at 7 DAA	Replicates	2	111.05	55.53	1.54^{ns}	3.25	5.21
	Treatments	19	9132.58	480.66	13.36^{**}	1.87	2.43
	Barnyardgrass (B)	1	1445.50	1445.50	40.19^{**}	4.10	7.35
	Doses (D)	9	7263.24	807.03	22.44^{**}	2.14	2.91
	B×D	9	423.83	47.09	1.31^{ns}	2.14	2.91
	Error	38	1366.79	35.97			
	Total	59	10610.42				
Number of seeds	Replicates	2	36853.03	18426.52	0.09^{ns}	3.25	5.21
	Treatments	19	42850051.52	2255265.87	10.93^{**}	1.87	2.43
	Barnyardgrass (B)	1	7671950.42	7671950.42	37.17^{**}	4.10	7.35
	Doses (D)	9	33153975.68	3683775.08	17.85^{**}	2.14	2.91
	B×D	9	2024125.42	224902.82	1.09^{ns}	2.14	2.91
	Error	38	7844023.63	206421.67			
	Total	59	50730928.18				
Dry weight (g)	Replicates	2	8.25	4.13	0.36^{ns}	3.25	5.21
	Treatments	19	2815.90	148.21	13.02^{**}	1.87	2.43
	Barnyardgrass (B)	1	621.85	621.85	54.64^{**}	4.10	7.35
	Doses (D)	9	1863.01	207.00	18.19^{**}	2.14	2.91
	B×D	9	331.05	36.78	3.23^{**}	2.14	2.91
	Error	38	432.50	11.38			
	Total	59	3256.65				

348 Note: the symbols * and ** indicate significant and highly significant effects based on DMRT at 1% and 5% levels,
349 respectively. ns= non significant and DAA= days after application.
350

351 Table 3. GR_{50} and resistance index of barnyardgrass to bispyribac-sodium herbicide.

Barnyardgrass	Regression equation	GR_{50} (g a.i.ha^{-1})	Resistance index
SB3 (resistant)	Y= -1.74 + 4.32X	305.36	9.82
MDN (susceptible)	Y= -4.46 + 6.65X	31.08	

352 Note: GR= growth reduction, X= LogDose, a.i.= active ingredient.



353 Figure 3. Dose-response testing of bispyribac-sodium herbicide on barnyardgrass: resistant-R (SB3) and susceptible-S (MDN). A= survival; B=
354 SPAD total chlorophyll; C= number of seeds; D= dry weight.

Experiment 3. Impact of Mixed Herbicides on Barnyardgrass Resistant Progenies

The effects of resistant-biotype progenies, mixed herbicides, and their interaction on the vegetative characteristics of bispyribac-sodium resistant barnyardgrass are shown in Table 4. The P3 biotype progeny had significantly higher weed height, leaf length and width with an increase of 30.83, 23.34, and 33.73%, respectively, compared to the P1 biotype progeny, but with a lower mortality rate. Similarly, the highest number of tillers was observed in the P3 biotype progeny, although the effect was not statistically significant. The mixed herbicide of fenoxaprop-P-ethyl+ethoxysulfuron (M3) significantly caused 100% mortality in the biotype progenies of bispyribac-sodium resistant barnyardgrass, preventing all vegetative attributes from being observed. The interaction of florpyrauxifen-benzyl+cyhalofop-butyl and fenoxaprop-P-ethyl+ethoxysulfuron effectively controlled the SB3 biotype progeny at both low and labeled-doses (P1M2, P1M3, P2M2, P2M3) up to 100%. However, 100% control of the high-dose SB3 biotype progeny was achieved only with the fenoxaprop-P-ethyl+ethoxysulfuron (P3M3).

The effects of resistant-biotype progenies, mixed herbicides, and their interaction on the physiological characteristics of bispyribac-sodium resistant barnyardgrass are shown in Figure 4 and Table 5. The SPAD total chlorophyll of all resistant-biotype progenies of barnyardgrass significantly decreased due to the mixed herbicides from 1 to 7 DAA, but the SB3 biotype progeny at the labeled (P2) and high-doses (P3) increased at 21 DAA. The highest SPAD total chlorophyll was found in the P3 biotype progeny with an increase of 47.93% at 21 DAA compared to the P1 biotype progeny. Similarly, the mixed herbicides of florpyrauxifen-benzyl+cyhalofop-butyl (M2) and fenoxaprop-P-ethyl+ethoxysulfuron (M3) significantly suppressed SPAD total chlorophyll from 1 to 21 DAA with the greatest decrease of 100% observed in M3 treatment. However, SPAD total chlorophyll increased when exposed to M1 and M4 treatments at 21 DAA. The interaction of florpyrauxifen-benzyl+cyhalofop-butyl and fenoxaprop-P-ethyl+ethoxysulfuron had a decrease in SPAD total chlorophyll in the SB3 biotype progeny at both low and labeled-dose (P1M2, P1M3, P2M2, P2M3) up to 100% at 21 DAA, although the effect was insignificant. A 100% control of the high-dose SB3 biotype progeny was only achieved with the use of fenoxaprop-P-ethyl+ethoxysulfuron (P3M3). Based on other physiological parameters, the P3 biotype progeny showed significantly higher stomatal conductance than the others. Likewise, the P3 biotype progeny showed higher transpiration rate, photosynthesis rate, and intercellular CO_2 concentration. Other results, fenoxaprop-P-ethyl+ethoxysulfuron (M3) significantly inhibited transpiration rate, photosynthesis rate, and stomatal conductance with the greatest inhibition among the mixed herbicides. However, all

mixed herbicides showed an increase in intercellular CO_2 concentration compared to the control. The interaction of low-dose SB3 biotype progeny with fenoxaprop-P-ethyl+ethoxysulfuron (P1M3) significantly inhibited stomatal conductance in bispyribac-sodium resistant barnyardgrass.

The effects of resistant-biotype progenies, mixed herbicides, and their interaction on the histological characteristics of bispyribac-sodium resistant barnyardgrass are shown in Table 6. The P3 biotype progeny significantly increased the size of the upper epidermis, mesophyll, and stomatal density by 8.03; 19.47; and 15.42%, respectively, compared to the P1 biotype progeny. Other results showed that the mixed herbicides of florpyrauxifen-benzyl+cyhalofop-butyl (M2) and fenoxaprop-P-ethyl+ethoxysulfuron (M3) significantly inhibited the size of the upper epidermis, mesophyll, lower epidermis, and stomatal density. The highest decrease in the histological tissue size of bispyribac-sodium resistant barnyardgrass was found in the M3 herbicide at 45.10; 50.39; 52.74; and 45.47% compared to the control. However, the histological tissue size increased upon exposure to cyhalofop-butyl+penoxsulam (M1) and bentazone+MCPA (M4). The interaction of low-dose SB3 biotype progeny with the fenoxaprop-P-ethyl+ethoxysulfuron (P1M3) significantly inhibited the histological tissue size of bispyribac-sodium resistant barnyardgrass.

The effects of resistant-biotype progenies, mixed herbicides, and their interaction on the yield attributes of bispyribac-sodium resistant barnyardgrass are shown in Table 7. The P3 biotype progeny significantly increased the dry weight per individual by 38.26% compared to the P1 biotype progeny. The flag leaf and panicle length of the P3 biotype progeny were higher, but also accelerated the flowering period. Other results, fenoxaprop-P-ethyl+ethoxysulfuron (M3) was 100% effective in suppressing the yield attributes of bispyribac-sodium resistant barnyardgrass compared to other mixed herbicides. Although the effect was insignificant, the interaction of florpyrauxifen-benzyl+cyhalofop-butyl and fenoxaprop-P-ethyl+ethoxysulfuron could inhibit the yield attributes of the SB3 biotype progeny at both low and labeled-doses (P1M2, P1M3, P2M2, P2M3) up to 100%. A 100% control of high-dose SB3 biotype progeny was achieved only with fenoxaprop-P-ethyl+ethoxysulfuron (P3M3).

Experiment 3. Impact of Mixed Herbicides on Irrigated Rice Plants

The increase in vegetative growth of irrigated rice plants due to resistant-biotype progenies, mixed herbicides, and their interaction is shown in Table 4. The number of tillers in irrigated rice grown simultaneously with the P3 biotype progeny was significantly inhibited by 11.61% compared to the P1 biotype progeny. However, biotype progenies had an insignificant effect on plant height and leaf area of irrigated rice plants. Mixed herbicides and their

interaction were insignificant effect on all vegetative growth of irrigated rice. Fenoxaprop-P-ethyl+ethoxysulfuron (M3) produced higher height and leaf area in irrigated rice plants than other mixed herbicides.

Physiological characteristics of irrigated rice plants, attributable to resistant-biotype progenies, mixed herbicides, and their interaction are shown in Figure 4 and Table 5. Irrigated rice plants grown simultaneously with the P3 biotype progeny showed inhibition of SPAD total chlorophyll at 7 DAA, transpiration rate, and intercellular CO_2 concentration were 4.05; 30.53; and 7.69%, respectively compared to the P1 biotype progeny. All mixed herbicides in this study significantly reduced SPAD total chlorophyll of irrigated rice at 1, 3, and 5 DAA relative to the pre-application. There was a recovery of SPAD total chlorophyll of irrigated rice plants at 7 DAA when exposed to cyhalofop-butyl+penoxsulam (M1) and fenoxaprop-P-ethyl+ethoxysulfuron (M3). At 21 DAA, recovery of SPAD total chlorophyll occurred with all herbicide mixtures, although the values were lower than those of the control. The lowest inhibition of SPAD total chlorophyll in irrigated rice plants was found with cyhalofop-butyl+penoxsulam (M1) by 6.84% at 21 DAA. Other physiological results showed that the photosynthesis rate of irrigated rice plants was significantly affected by fenoxaprop-P-ethyl+ethoxysulfuron (M3), increasing by 7.23% relative to the control. The interaction of biotype progenies with mixed herbicides (P×M) significantly inhibited SPAD total chlorophyll at 3, 5, 7, and 21 DAA, but had an insignificant effect on other photosynthesis indicators. The low-dose SB3 biotype progeny without herbicide spray (P1M0) had the highest SPAD total chlorophyll of irrigated rice plants by 53.70 at 21 DAA compared to other interactions.

The leaf histological characteristics of irrigated rice affected by resistant-biotype progenies, mixed herbicides, and their interaction are shown in Table 6. Biotype progenies only significantly affected the upper epidermal tissue size of irrigated rice plants, but had an insignificant effect on other histological tissues. The development of upper epidermal tissue in irrigated rice plants grown simultaneously with the P3 biotype progeny was inhibited by 16.31% compared to the P1 biotype progeny. Mixed herbicides and their interaction had an insignificant effect on all histological tissues. However, all mixed herbicides increased the size of the upper epidermis, mesophyll, lower epidermis, and stomatal density of irrigated rice.

The yield attributes of irrigated rice plants affected by resistant-biotype progenies, mixed herbicides, and their interaction are shown in Table 8. Biotype progenies had an insignificant effect on all yield attributes of irrigated rice. However, the irrigated rice plants grown simultaneously with the P3 biotype progeny showed the lowest yield attributes and prolonged flowering period compared to the P1 biotype progeny. The dominant mixed

herbicides significantly affected the yield attributes of irrigated rice plants, except for 1000-grains weight and harvest index. Fenoxaprop-P-ethyl+ethoxysulfuron (M3) was shown to increase the number of productive tillers, flag leaf area, dry weight, panicle length, number and weight of filled grains, and productivity of irrigated rice plants by 10.57; 18.19; 30.40; 6.20; 43.73; 61.21; and 27.01%, respectively compared to the control. Similarly, the M3 herbicide accelerated flowering at 69.56 days. The interaction between biotype progenies and mixed herbicides only significantly affected the number of productive tillers in irrigated rice plants, but had an insignificant effect on other yield attributes.

The mixed herbicides significantly affected the amylose content of irrigated rice grains, but the biotype progenies and their interaction were insignificant (Figure 5). The lowest amylose content was found in irrigated rice plants grown simultaneously with the P3 biotype progeny. The use of florpyrauxifen-benzyl+cyhalofop-butyl (M2) and fenoxaprop-P-ethyl+ethoxysulfuron (M3) significantly increased the amylose content compared to the control, but cyhalofop-butyl+penoxsulam (M1) and bentazone+MCPA (M4) caused a decrease. The highest increase in amylose content of 31.50% (moderate category) was found with the M3 herbicide. Although the effect was insignificant, the interaction of low-dose SB3 biotype progeny with fenoxaprop-P-ethyl+ethoxysulfuron (P1M3) produced the highest amylose content compared to other interactions.

The correlation between histophysiological and yield attributes of irrigated rice plants is shown in Table 9. The upper epidermis and weight of filled grains were significantly positively correlated on the productivity of irrigated rice plants with values of 0.348 and 0.818. This was because the upper epidermis played an important role in increasing intercellular CO_2 concentration (0.339), which in turn enhanced photosynthesis rate (0.295 and 0.489) and contributed to the accumulation of dry weight. The increase in dry weight, stomatal density, and number of productive tillers significantly affected the weight of filled grains with correlation values of 0.424; 0.314; and 0.373, respectively. The leaves histological characteristics, such as the upper epidermis and stomatal influenced CO_2 exchange and photosynthesis activity, thereby affecting the productivity of irrigated rice plants. Among the histophysiological variables, only stomatal conductance showed a positive, significant correlation (0.380) with amylose content in irrigated rice grains. This indicates that higher stomatal conductance can increase the photosynthesis rate, which is crucial for grain filling and starch synthesis in irrigated rice.

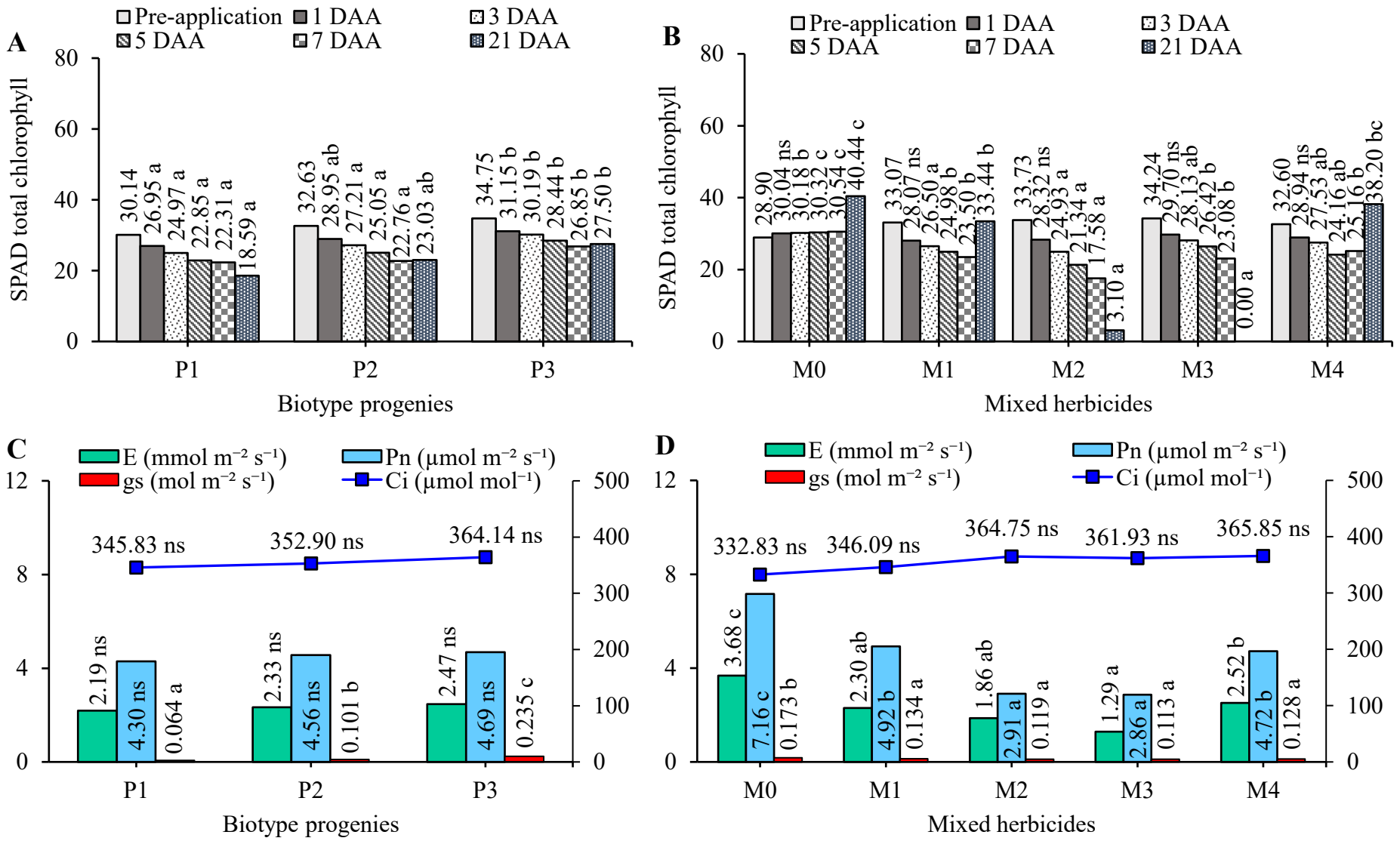
The weed control efficiency (WCE) and the yield increase over control (YOC) in irrigated rice resulting from mixed herbicides are shown in Figure 6. Fenoxaprop-P-

ethyl+ethoxysulfuron (M3) had the highest WCE and YOC at 100 and 27.01%, respectively, followed by florpyrauxifen-benzyl+cyhalofop-butyl (M2). Therefore, both mixed herbicides (M2 and M3) effectively controlled bispyribac-sodium resistant barnyardgrass (SB3 biotype) and positively impacted irrigated rice productivity. However, cyhalofop-butyl+penoxsulam (M1) and bentazone+MCPA (M4) were less effective.

Table 4. Vegetative traits of bispyribac-sodium resistant barnyardgrass and irrigated rice as affected by biotype progenies, mixed herbicides, and their interactions.

Treatments		Resistant barnyardgrass								Irrigated rice					
		Mortality (%) 21 DAA	Weed height (cm)		Leaf length (cm)		Leaf width (cm)		Number of tillers		Plant height (cm)		Leaf area (cm^2)		Number of tillers	
			PA	21 DAA	PA	21 DAA	PA	21 DAA	PA	21 DAA	PA	Increase (21 DAA)	PA	Increase (21 DAA)	PA	Increase (21 DAA)
Biotype progenies (P)																
P1		48.00 a	15.69	32.24 a	15.20	20.61 a	0.327	0.501 a	0.80	1.73 ns	21.15	39.38 ns	8.20	31.62 ns	1.93	40.13 a
P2		41.33 b	18.32	37.14 b	17.26	22.55 ab	0.509	0.648 b	1.33	3.27 ns	20.17	38.57 ns	8.20	31.13 ns	2.07	38.67 ab
P3		38.67 b	19.40	42.18 c	16.60	25.42 b	0.542	0.670 b	1.80	4.20 ns	19.38	38.65 ns	7.79	30.59 ns	1.73	35.47 b
Mixed herbicides (M)																
M0		0.00 c	16.62	67.41 c	15.79	37.16 c	0.452	1.001 b	0.78	3.44 b	21.21	37.98 ns	7.17	27.71 ns	1.89	37.33 ns
M1		15.56 b	16.99	42.28 b	15.64	29.73 b	0.436	1.007 b	1.44	8.00 c	20.04	38.74 ns	8.05	31.54 ns	1.89	40.33 ns
M2		97.78 a	17.74	1.63 a	15.96	0.96 a	0.499	0.072 a	1.56	0.00 a	19.74	39.68 ns	7.39	32.23 ns	1.67	35.89 ns
M3		100.00 a	19.41	0.00 a	17.64	0.00 a	0.438	0.000 a	1.78	0.00 a	20.77	40.02 ns	9.46	34.47 ns	1.89	38.22 ns
M4		0.00 c	18.26	74.61 d	16.73	46.46 d	0.471	0.951 b	1.00	3.89 b	19.40	37.91 ns	8.24	29.62 ns	2.22	38.67 ns
Interactions (P×M)																
P1	M0	0.00 c	15.50	60.67 d	16.00	34.87 ns	0.355	0.907 ns	0.67	2.33 ns	22.47	37.83 ns	7.77	27.83 ns	2.00	39.67 ns
	M1	40.00 b	15.20	35.80 b	13.30	24.33 ns	0.268	0.837 ns	0.67	4.33 ns	20.90	39.70 ns	9.48	30.02 ns	2.00	38.00 ns
	M2	100.00 a	17.03	0.00 a	16.70	0.00 ns	0.474	0.000 ns	1.00	0.00 ns	19.47	40.70 ns	7.08	32.42 ns	1.67	37.67 ns
	M3	100.00 a	15.33	0.00 a	15.10	0.00 ns	0.218	0.000 ns	1.00	0.00 ns	21.77	41.13 ns	8.89	37.56 ns	1.67	41.33 ns
	M4	0.00 c	15.40	64.73 de	14.90	43.83 ns	0.319	0.760 ns	0.67	2.00 ns	21.13	37.53 ns	7.77	30.25 ns	2.33	44.00 ns
P2	M0	0.00 c	14.77	68.80 def	15.20	36.73 ns	0.474	1.058 ns	0.67	4.00 ns	21.87	38.80 ns	7.12	32.27 ns	2.00	39.67 ns
	M1	6.67 c	15.83	40.77 b	16.50	28.77 ns	0.459	1.086 ns	1.67	8.00 ns	20.30	38.87 ns	7.69	30.20 ns	2.33	45.33 ns
	M2	100.00 a	16.83	0.00 a	14.20	0.00 ns	0.451	0.000 ns	1.33	0.00 ns	20.13	38.80 ns	7.22	29.92 ns	1.67	34.33 ns
	M3	100.00 a	24.63	0.00 a	22.63	0.00 ns	0.627	0.000 ns	2.00	0.00 ns	21.33	37.50 ns	11.13	30.49 ns	2.00	35.00 ns
	M4	0.00 c	19.53	76.13 fg	17.77	47.27 ns	0.532	1.098 ns	1.00	4.33 ns	17.23	38.87 ns	7.83	32.75 ns	2.33	39.00 ns
P3	M0	0.00 c	19.60	72.77 ef	16.17	39.87 ns	0.526	1.038 ns	1.00	4.00 ns	19.30	37.30 ns	6.62	23.03 ns	1.67	32.67 ns
	M1	0.00 c	19.93	50.27 c	17.13	36.10 ns	0.580	1.098 ns	2.00	11.67 ns	18.93	37.67 ns	6.96	34.40 ns	1.33	37.67 ns
	M2	93.33 a	19.37	4.90 a	16.97	2.88 ns	0.572	0.215 ns	2.33	0.00 ns	19.63	39.53 ns	7.88	34.34 ns	1.67	35.67 ns
	M3	100.00 a	18.27	0.00 a	15.20	0.00 ns	0.468	0.000 ns	2.33	0.00 ns	19.20	41.43 ns	8.37	35.35 ns	2.00	38.33 ns
	M4	0.00 c	19.83	82.97 g	17.53	48.27 ns	0.562	0.996 ns	1.33	5.33 ns	19.83	37.33 ns	9.13	25.85 ns	2.00	33.00 ns
CV (%)		15.85	-	13.80	-	18.47	-	19.70	-	24.15	-	5.68	-	15.45	-	7.34

Note: values within a column followed by different letters are significantly different based on DMRT at the 5% level. Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. PA= pre-application, ns= not significant, CV= coefficient of variation, and DAA= days after application.


A
Pre-application
1 DAA
3 DAA
5 DAA
7 DAA
21 DAA
SPAD total chlorophyll
0
20
40
60
80
30.14
26.95 a
24.97 a
22.85 a
22.31 a
18.59 a
32.63
28.95 ab
27.21 a
25.05 a
22.76 a
23.03 ab
34.75
31.15 b
30.19 b
28.44 b
26.85 b
27.50 b
P1
P2
P3
Biotype progenies
B
Pre-application
1 DAA
3 DAA
5 DAA
7 DAA
21 DAA
SPAD total chlorophyll
0
20
40
60
80
28.90
30.04 ns
30.18 b
30.32 c
30.54 c
40.44 c
33.07
28.07 ns
26.50 a
24.98 b
23.50 b
33.44 b
33.73
28.32 ns
24.93 a
21.34 a
17.58 a
3.10 a
34.24
29.70 ns
28.13 ab
26.42 b
23.08 b
0.00 a
32.60
28.94 ns
27.53 ab
24.16 ab
25.16 b
38.20 bc
M0
M1
M2
M3
M4
Mixed herbicides
C
E (mmol m⁻² s⁻¹)
Pn (µmol m⁻² s⁻¹)
gs (mol m⁻² s⁻¹)
Ci (µmol mol⁻¹)
0
4
8
12
0
100
200
300
400
500
345.83 ns
352.90 ns
364.14 ns
2.19 ns
4.30 ns
0.064 a
2.33 ns
4.56 ns
0.101 b
2.47 ns
4.69 ns
0.235 c
P1
P2
P3
Biotype progenies
D
E (mmol m⁻² s⁻¹)
Pn (µmol m⁻² s⁻¹)
gs (mol m⁻² s⁻¹)
Ci (µmol mol⁻¹)
0
4
8
12
0
100
200
300
400
500
332.83 ns
346.09 ns
364.75 ns
361.93 ns
365.85 ns
3.68 c
7.16 c
0.173 b
2.30 ab
4.92 b
0.134 a
1.86 ab
2.91 a
0.119 a
1.29 a
2.86 a
0.113 a
2.52 b
4.72 b
0.128 a
M0
M1
M2
M3
M4
Mixed herbicides

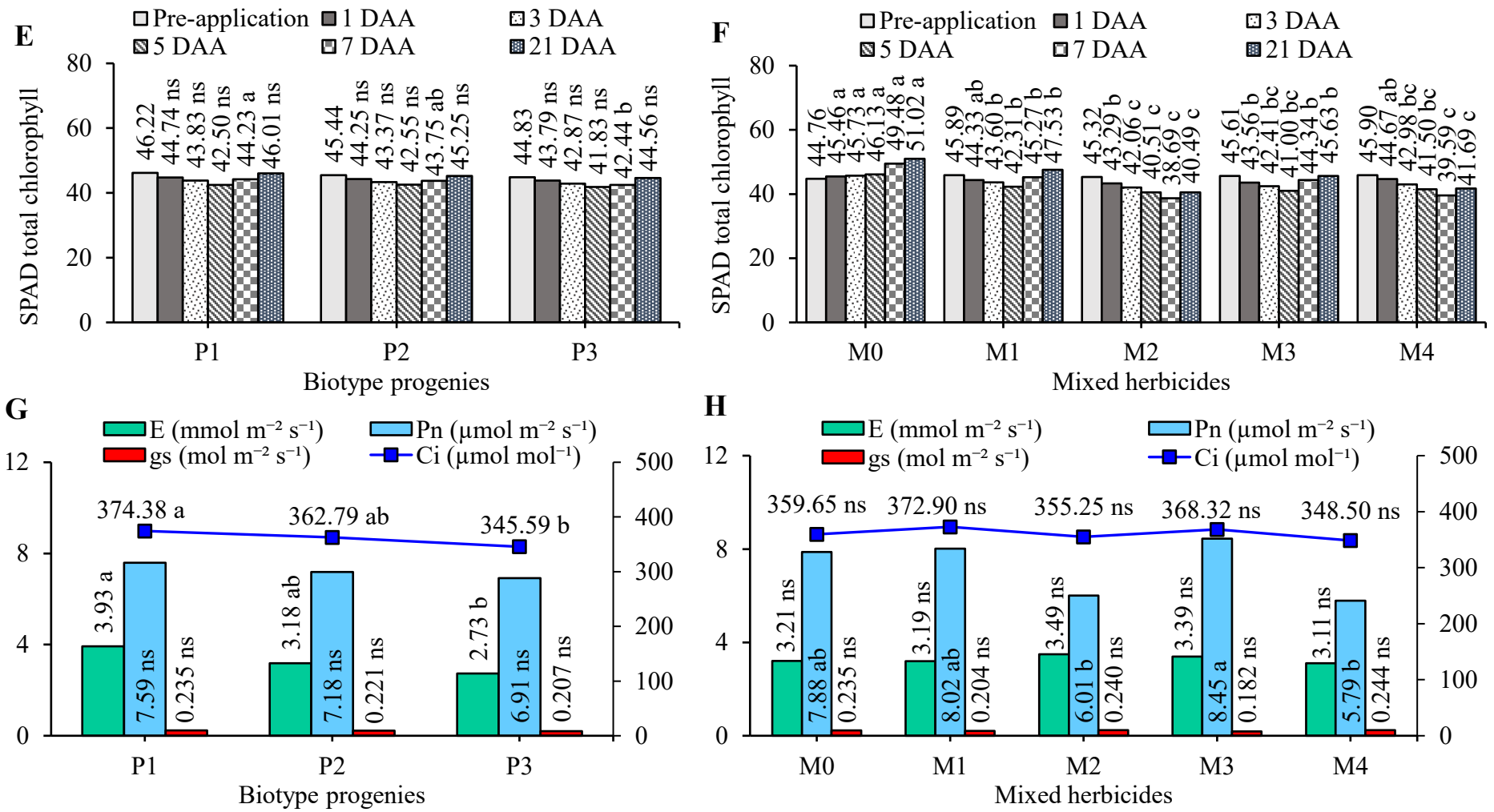


Figure 4. Physiological traits of bispyribac-sodium resistant barnyardgrass (A-D) and irrigated rice (E-H) as affected by biotype progenies and mixed herbicides. Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. Photosynthesis parameters: photosynthesis rate (P_n), transpiration rate (E), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s). ns= not significant and DAA= days after application.

Table 5. Interactions of biotype progenies and mixed herbicides on the physiological traits of bispyribac-sodium resistant barnyardgrass and irrigated rice.

Species	Interactions (P×M)		Physiological traits									
			SPAD total chlorophyll						P_n	E	C_i	g_s
			Pre-application	1 DAA	3 DAA	5 DAA	7 DAA	21 DAA	(μmol m^{-2} s^{-1})	(mmol m^{-2} s^{-1})	(μmol mol^{-1})	(mol m^{-2} s^{-1})
Resistant barnyardgrass	P1	M0	26.80	28.27 ns	28.37 ns	28.50 ns	28.77 ns	37.17 ns	5.42 ns	3.09 ns	317.32 ns	0.076 abc
		M1	30.57	25.37 ns	24.27 ns	23.03 ns	21.17 ns	23.40 ns	4.89 ns	2.60 ns	342.29 ns	0.066 abc
		M2	29.70	26.77 ns	22.67 ns	20.53 ns	17.97 ns	0.00 ns	2.88 ns	1.76 ns	358.81 ns	0.047 ab
		M3	31.67	27.17 ns	24.07 ns	20.77 ns	19.07 ns	0.00 ns	2.82 ns	0.82 ns	359.53 ns	0.044 a
		M4	31.97	27.17 ns	25.50 ns	21.40 ns	24.57 ns	32.37 ns	5.47 ns	2.70 ns	351.20 ns	0.089 c
	P2	M0	29.13	29.67 ns	29.83 ns	30.00 ns	30.17 ns	38.40 ns	7.41 ns	3.29 ns	324.03 ns	0.194 d
		M1	33.03	27.63 ns	25.83 ns	23.70 ns	21.90 ns	37.23 ns	4.42 ns	2.28 ns	336.33 ns	0.080 abc
		M2	30.93	26.53 ns	21.90 ns	17.97 ns	14.10 ns	0.00 ns	3.29 ns	1.73 ns	364.49 ns	0.075 abc
		M3	36.47	30.90 ns	29.73 ns	28.67 ns	23.07 ns	0.00 ns	3.31 ns	1.68 ns	365.47 ns	0.069 abc
		M4	33.60	30.03 ns	28.73 ns	24.90 ns	24.57 ns	39.50 ns	4.37 ns	2.67 ns	374.17 ns	0.086 bc
	P3	M0	30.77	32.20 ns	32.33 ns	32.47 ns	32.70 ns	45.77 ns	8.65 ns	4.67 ns	357.15 ns	0.250 f
		M1	35.60	31.20 ns	29.40 ns	28.20 ns	27.43 ns	39.70 ns	5.46 ns	2.03 ns	359.65 ns	0.255 f
		M2	40.57	31.67 ns	30.23 ns	25.53 ns	20.67 ns	9.30 ns	2.55 ns	2.10 ns	370.94 ns	0.236 ef
		M3	34.60	31.03 ns	30.60 ns	29.83 ns	27.10 ns	0.00 ns	2.46 ns	1.36 ns	360.78 ns	0.225 def
		M4	32.23	29.63 ns	28.37 ns	26.17 ns	26.33 ns	42.73 ns	4.33 ns	2.19 ns	372.19 ns	0.209 de
	CV (%)		-	10.93	12.38	13.98	14.82	18.81	21.22	18.79	8.16	15.50
Irrigated rice	P1	M0	45.37	46.33 ns	46.53 ab	46.90 a	52.67 a	53.70 a	8.36 ns	4.24 ns	338.46 ns	0.213 ns
		M1	47.03	43.80 ns	43.03 cde	41.80 b-e	43.70 cde	46.87 cd	8.68 ns	4.45 ns	387.36 ns	0.197 ns
		M2	45.87	43.40 ns	42.00 cde	40.33 de	38.93 fg	40.00 f	5.87 ns	4.05 ns	381.75 ns	0.304 ns
		M3	47.17	45.03 ns	44.00 bcd	42.47 bcd	46.93 bc	48.80 bc	8.98 ns	3.68 ns	382.89 ns	0.164 ns
		M4	45.67	45.13 ns	43.57 cd	41.00 cde	38.93 fg	40.70 f	6.07 ns	3.21 ns	381.44 ns	0.297 ns
	P2	M0	46.10	46.53 ns	46.80 a	47.17 a	49.87 ab	51.23 ab	8.22 ns	2.53 ns	377.30 ns	0.242 ns
		M1	45.57	44.77 ns	44.10 bcd	43.57 bc	46.70 bc	47.47 bc	7.47 ns	2.40 ns	373.61 ns	0.231 ns
		M2	45.80	44.20 ns	43.37 cde	41.57 cde	39.33 fg	41.67 ef	5.97 ns	3.54 ns	355.93 ns	0.209 ns
		M3	45.30	42.70 ns	41.87 de	40.40 de	44.17 cde	45.03 cde	8.03 ns	3.83 ns	382.15 ns	0.217 ns
		M4	44.43	43.07 ns	40.70 e	40.03 de	38.67 fg	40.83 f	6.21 ns	3.62 ns	324.96 ns	0.205 ns
	P3	M0	42.80	43.50 ns	43.87 cd	44.33 b	45.90 c	48.13 bc	7.05 ns	2.85 ns	363.17 ns	0.251 ns
		M1	45.07	44.43 ns	43.67 cd	41.57 cde	45.40 cd	48.27 bc	7.90 ns	2.74 ns	357.73 ns	0.183 ns
		M2	44.30	42.27 ns	40.80 e	39.63 e	37.80 g	39.80 f	6.20 ns	2.88 ns	328.07 ns	0.207 ns
		M3	44.37	42.93 ns	41.37 de	40.13 de	41.93 def	43.07 def	8.33 ns	2.65 ns	339.91 ns	0.166 ns
		M4	47.60	45.80 ns	44.67 abc	43.47 bc	41.17 efg	43.53 def	5.09 ns	2.51 ns	339.10 ns	0.229 ns
	CV (%)			-	3.44	3.25	3.32	4.60	4.74	19.47	21.68	9.10

Note: values within a column followed by different letters are significantly different based on DMRT at the 5% level. Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. Photosynthesis parameters: photosynthesis rate (P_n), transpiration rate (E), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s). ns= not significant, CV= coefficient of variation, and DAA= days after application.

Table 6. Histological traits of bispyribac-sodium resistant barnyardgrass and irrigated rice as affected by biotype progenies, mixed herbicides, and their interactions.

Treatments		Resistant barnyardgrass				Irrigated rice			
		Upper epidermis (μm)	Mesophyll (μm)	Lower epidermis (μm)	Stomatal density (n/μm)	Upper epidermis (μm)	Mesophyll (μm)	Lower epidermis (μm)	Stomatal density (n/μm)
Biotype progenies (P)									
P1		25.54 a	96.18 a	19.25 ns	87.76 a	22.25 b	130.86 ns	16.64 ns	35.83 ns
P2		27.83 b	103.63 b	20.30 ns	95.34 b	21.68 ab	134.00 ns	16.51 ns	36.68 ns
P3		27.59 b	114.91 c	20.77 ns	101.29 c	18.62 a	140.69 ns	18.29 ns	38.61 ns
Mixed herbicides (M)									
M0		27.45 b	112.78 c	21.90 c	100.78 b	19.48 ns	131.15 ns	15.76 ns	34.23 ns
M1		34.48 c	130.70 d	23.85 d	105.96 b	21.64 ns	139.39 ns	18.15 ns	38.84 ns
M2		16.51 a	69.94 b	13.55 b	60.37 a	19.66 ns	132.24 ns	16.96 ns	36.37 ns
M3		15.07 a	55.95 a	10.35 a	54.96 a	23.71 ns	140.25 ns	18.32 ns	39.89 ns
M4		41.43 d	155.15 e	30.89 e	151.91 c	19.75 ns	132.90 ns	16.54 ns	35.87 ns
Interactions (P×M)									
P1	M0	24.30 d	99.60 d	19.72 de	100.33 cd	19.66 ns	115.92 ns	14.74 ns	34.19 ns
	M1	34.89 gh	128.45 fgh	25.23 fg	97.40 c	24.56 ns	134.42 ns	17.42 ns	36.88 ns
	M2	17.23 c	62.18 ab	13.55 bc	65.22 b	19.51 ns	131.51 ns	18.19 ns	41.18 ns
	M3	12.63 a	51.04 a	9.37 a	40.61 a	26.20 ns	142.56 ns	17.73 ns	37.18 ns
	M4	38.65 hi	139.61 gh	28.39 h	135.24 e	21.30 ns	129.91 ns	15.10 ns	29.73 ns
P2	M0	28.11 e	112.85 de	23.45 f	95.41 c	20.09 ns	133.02 ns	16.69 ns	27.70 ns
	M1	32.83 fg	122.57 ef	26.94 gh	107.79 cd	21.52 ns	149.86 ns	19.16 ns	38.25 ns
	M2	19.22 c	78.05 c	12.28 abc	59.11 b	20.15 ns	127.50 ns	15.34 ns	38.85 ns
	M3	16.88 bc	61.43 ab	11.33 ab	62.87 b	25.10 ns	127.54 ns	15.11 ns	38.62 ns
	M4	42.13 ij	143.26 h	27.51 gh	151.52 f	21.52 ns	132.08 ns	16.23 ns	39.97 ns
P3	M0	29.93 ef	125.89 efg	22.53 ef	106.60 cd	18.69 ns	144.51 ns	15.86 ns	40.81 ns
	M1	35.71 gh	141.07 gh	19.38 d	112.70 d	18.83 ns	133.90 ns	17.87 ns	41.39 ns
	M2	13.09 ab	69.60 bc	14.81 c	56.77 b	19.31 ns	137.70 ns	17.35 ns	29.09 ns
	M3	15.71 abc	55.39 ab	10.36 a	61.39 b	19.84 ns	150.65 ns	22.11 ns	43.88 ns
	M4	43.51 j	182.58 i	36.77 i	168.97 g	16.42 ns	136.70 ns	18.27 ns	37.91 ns
CV (%)		8.38	8.04	8.46	8.47	16.71	18.79	19.55	14.21

Note: values within a column followed by different letters are significantly different based on DMRT at the 5% level. Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. ns= not significant and CV= coefficient of variation.

Table 7. Yield attributes of bispyribac-sodium resistant barnyardgrass as affected by biotype progenies, mixed herbicides, and their interactions.

Treatments		Yield attributes of resistant barnyardgrass					
		Flowering age (days)	Flag leaf		Panicle length (cm)	Number of seeds	Dry weight per individual (g)
			Length (cm)	Width (mm)			
Biotype progenies (P)							
P1		43.67 ns	11.45 ns	7.14 ns	8.65 ns	8,505 ns	8.18 a
P2		40.73 ns	11.21 ns	7.15 ns	8.86 ns	11,057 ns	11.02 ab
P3		38.73 ns	15.62 ns	8.36 ns	9.49 ns	10,436 ns	11.31 b
Mixed herbicides (M)							
M0		53.78 b	23.07 c	12.88 c	15.63 c	17,272 c	20.66 c
M1		78.78 c	14.61 b	10.34 b	12.14 b	10,745 b	11.08 b
M2		8.78 a	1.97 a	1.03 a	1.17 a	478 a	1.42 a
M3		0.00 a	0.00 a	0.00 a	0.00 a	0 a	0.00 a
M4		63.89 b	24.17 c	13.52 c	16.06 c	21,504 c	17.69 c
Interactions (P×M)							
P1	M0	54.33 ns	26.00 ns	13.00 ns	16.26 ns	14,203 ns	15.76 ns
	M1	86.33 ns	14.65 ns	10.05 ns	11.66 ns	10,355 ns	9.86 ns
	M2	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0 ns	0.00 ns
	M3	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0 ns	0.00 ns
	M4	77.67 ns	16.60 ns	12.66 ns	15.34 ns	17,968 ns	15.31 ns
P2	M0	58.00 ns	17.30 ns	12.22 ns	14.74 ns	20,516 ns	24.41 ns
	M1	83.67 ns	14.30 ns	9.76 ns	12.53 ns	12,149 ns	9.92 ns
	M2	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0 ns	0.00 ns
	M3	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0 ns	0.00 ns
	M4	62.00 ns	24.47 ns	13.78 ns	17.01 ns	22,622 ns	20.75 ns
P3	M0	49.00 ns	25.90 ns	13.41 ns	15.90 ns	17,097 ns	21.81 ns
	M1	66.33 ns	14.87 ns	11.21 ns	12.22 ns	9,730 ns	13.47 ns
	M2	26.33 ns	5.90 ns	3.08 ns	3.50 ns	1,434 ns	4.27 ns
	M3	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0 ns	0.00 ns
	M4	52.00 ns	31.43 ns	14.12 ns	15.83 ns	23,921 ns	17.02 ns
CV (%)		22.18	29.47	22.31	23.18	28.34	23.65

Note: values within a column followed by different letters are significantly different based on DMRT at the 5% level. Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. ns= not significant and CV= coefficient of variation.

Table 8. Yield attributes of irrigated rice as affected by biotype progenies, mixed herbicides, and their interactions.

Treatments		Yield attributes of irrigated rice									
		Number of productive tillers	Flowering age (days)	Flag leaf area (cm^2)	Dry weight (g)	Panicle length (cm)	Number of filled grains	Weight of filled grains (g)	1000-grains weight (g)	Harvest index (%)	Productivity (ton ha^{-1})
Biotype progenies (P)											
P1		33.73 ns	70.40 ns	26.94 ns	263.52 ns	22.73 ns	3,731.07 ns	80.13 ns	22.73 ns	24.49 ns	5.48 ns
P2		34.07 ns	70.07 ns	25.87 ns	224.90 ns	22.32 ns	3,687.67 ns	79.18 ns	21.18 ns	26.34 ns	5.46 ns
P3		32.33 ns	70.73 ns	24.58 ns	220.73 ns	21.99 ns	3,271.87 ns	77.20 ns	20.92 ns	22.99 ns	4.94 ns
Mixed herbicides (M)											
M0		32.56 b	70.89 b	25.07 b	222.74 bc	22.76 ab	3,374.56 b	72.93 bc	20.76 ns	24.80 ns	5.11 ab
M1		32.33 b	70.22 ab	25.62 b	252.57 ab	21.09 bc	3,423.33 b	73.24 bc	21.31 ns	22.47 ns	5.26 ab
M2		33.33 b	70.33 ab	24.54 b	230.28 abc	23.11 a	3,738.78 b	85.20 ab	21.62 ns	25.37 ns	5.47 ab
M3		36.00 a	69.56 a	29.63 a	290.45 a	24.17 a	4,850.22 a	117.57 a	24.09 ns	29.21 ns	6.49 a
M4		32.67 b	71.00 b	24.13 b	185.88 c	20.60 c	2,430.78 c	46.81 c	20.28 ns	21.20 ns	4.13 b
Interactions (P×M)											
P1	M0	32.33 bcd	71.00 ns	28.21 ns	254.35 ns	22.83 ns	3,605.00 ns	81.91 ns	19.67 ns	21.93 ns	5.20 ns
	M1	30.00 cd	69.67 ns	26.02 ns	286.34 ns	20.80 ns	3,526.67 ns	73.47 ns	23.47 ns	22.60 ns	5.43 ns
	M2	32.00 bcd	70.67 ns	25.72 ns	233.06 ns	23.60 ns	3,337.33 ns	65.51 ns	24.03 ns	25.58 ns	5.36 ns
	M3	40.00 a	69.33 ns	30.37 ns	315.04 ns	24.93 ns	5,013.00 ns	122.01 ns	23.83 ns	27.55 ns	6.55 ns
	M4	34.33 bcd	71.33 ns	24.39 ns	228.83 ns	21.47 ns	3,173.33 ns	57.76 ns	22.67 ns	24.79 ns	4.88 ns
P2	M0	33.67 bcd	71.00 ns	21.59 ns	216.56 ns	22.70 ns	3,390.00 ns	74.29 ns	20.53 ns	26.48 ns	5.14 ns
	M1	35.33 abc	70.00 ns	26.68 ns	247.65 ns	20.47 ns	3,813.00 ns	79.74 ns	20.00 ns	24.61 ns	5.81 ns
	M2	32.33 bcd	69.33 ns	25.90 ns	227.20 ns	23.10 ns	4,092.33 ns	90.37 ns	21.73 ns	28.35 ns	5.89 ns
	M3	35.00 bcd	69.00 ns	29.28 ns	253.38 ns	24.50 ns	4,704.67 ns	114.45 ns	24.20 ns	31.40 ns	6.42 ns
	M4	34.00 bcd	71.00 ns	25.91 ns	179.72 ns	20.83 ns	2,438.33 ns	41.74 ns	19.43 ns	20.88 ns	4.02 ns
P3	M0	31.67 bcd	70.67 ns	25.40 ns	197.31 ns	22.73 ns	3,128.67 ns	62.57 ns	22.07 ns	26.00 ns	4.98 ns
	M1	31.67 bcd	71.00 ns	24.16 ns	223.73 ns	22.00 ns	2,930.33 ns	66.52 ns	20.47 ns	20.20 ns	4.54 ns
	M2	35.67 ab	71.00 ns	22.00 ns	230.58 ns	22.63 ns	3,786.67 ns	99.72 ns	19.10 ns	22.17 ns	5.16 ns
	M3	33.00 bcd	70.33 ns	29.23 ns	302.92 ns	23.07 ns	4,833.00 ns	116.25 ns	24.23 ns	28.67 ns	6.50 ns
	M4	29.67 d	70.67 ns	22.09 ns	149.10 ns	19.50 ns	1,680.67 ns	40.92 ns	18.73 ns	17.94 ns	3.50 ns
CV (%)		8.17	1.62	15.07	26.95	7.82	25.88	24.31	21.89	19.46	17.63

Note: values within a column followed by different letters are significantly different based on DMRT at the 5% level. Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. ns= not significant and CV= coefficient of variation.

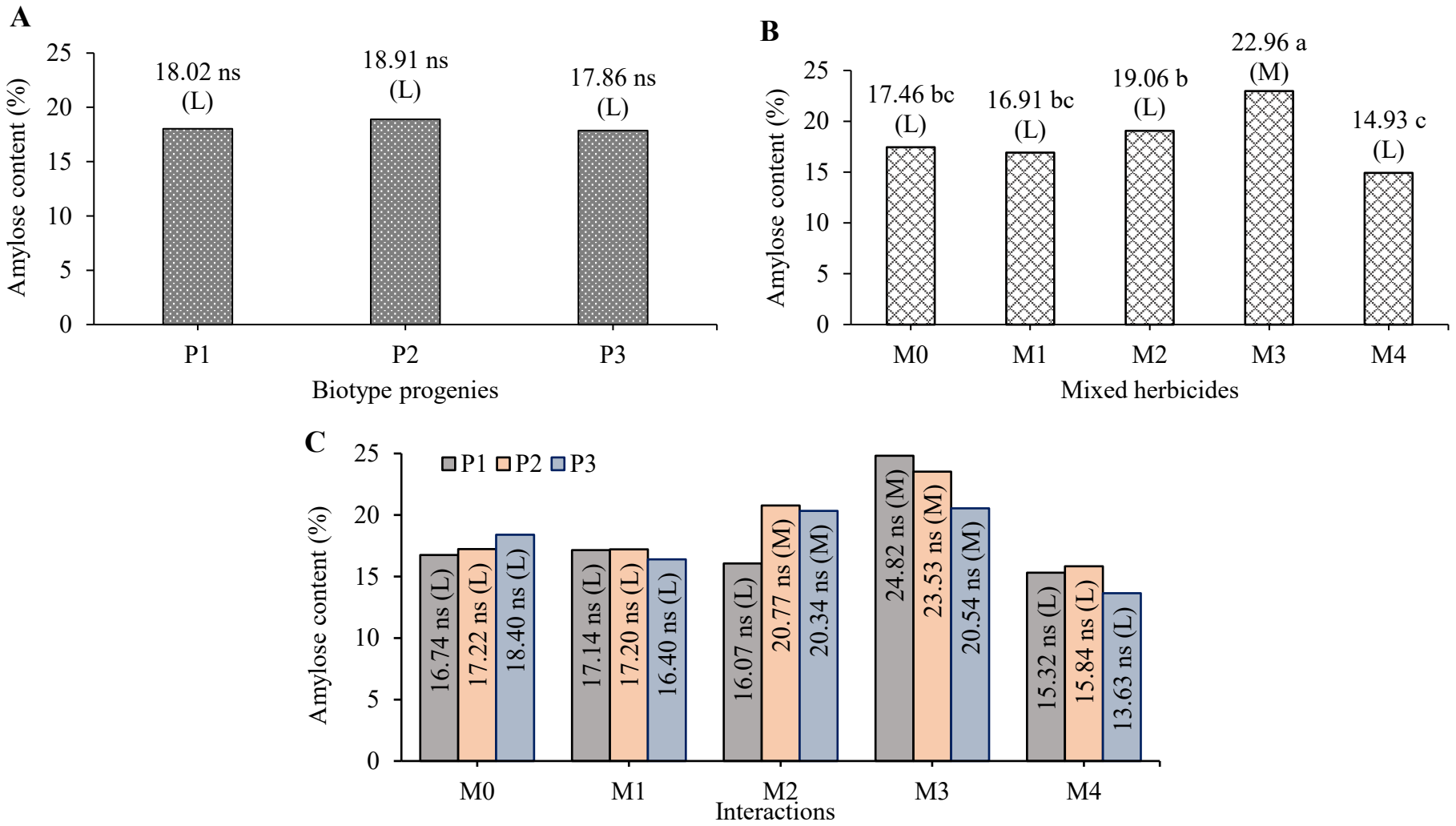


Figure 5. Amylose content of irrigated rice grains as caused by biotype progenies (A), mixed herbicides (B), and their interactions (C). Biotype progenies: P1=SB3 low-dose (1/16-fold), P2=SB3 labeled dose (1-fold), P3= SB3 high-dose (8-fold). Mixed herbicides: M0= unsprayed; M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; M4= bentazone+MCPA. The amylose content is classified as very low/VL (less than 10%), low/L (10–19.99%), moderate/M (20–25%), and high/H (greater than 25%). ns= not significant.

Table 9. Correlation values between irrigated rice characteristics caused by biotype progenies, mixed herbicides, and their interactions.

No	Characteristics of irrigated rice	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	UE	1	-0.028	-0.297*	0.230	0.210	0.181	-0.096	0.339*	-0.088	0.152	0.097	0.124	0.157	0.075	0.239	0.348*	0.223
2	M		1	0.151	0.065	-0.172	0.057	0.084	0.028	0.049	0.327*	0.032	0.044	0.195	-0.053	-0.063	0.032	0.013
3	LE			1	-0.106	0.072	-0.053	0.131	-0.050	0.029	0.028	-0.022	0.145	0.124	0.169	0.183	0.263	0.003
4	SD				1	0.051	-0.182	-0.068	0.124	-0.054	0.018	0.111	-0.087	0.030	-0.053	0.314*	0.288	0.054
5	PR					1	0.025	0.078	-0.051	0.251	0.083	0.232	0.147	0.339*	0.489**	0.251	0.218	0.052
6	TR						1	0.134	-0.016	0.034	0.110	-0.121	0.024	0.460**	0.030	-0.012	-0.061	0.076
7	SC							1	-0.002	-0.106	0.046	-0.190	-0.084	0.092	-0.085	0.246	0.253	0.380*
8	ICC								1	0.071	0.130	0.037	0.050	0.127	0.295*	0.264	0.218	0.257
9	STC									1	-0.069	-0.075	0.016	0.125	0.152	0.088	0.025	0.035
10	PH										1	0.209	0.113	0.159	0.441**	-0.086	0.017	0.097
11	LA											1	0.295*	0.061	0.218	0.151	0.136	0.183
12	NPT												1	0.177	0.226	0.373*	0.234	0.497**
13	FLA													1	0.265	0.247	0.085	0.079
14	DW														1	0.424**	0.233	0.275
15	WFG															1	0.818**	0.410**
16	P																1	0.349*
17	AC																	1

Note: the symbols * and ** indicate that the correlation is significant at the 0.05 and 0.01 levels. n= 45 samples. UE= upper epidermis, M= mesophyll, LE= lower epidermis, SD= stomatal density, PR= photosynthesis rate, TR= transpiration rate, SC= stomatal conductance, ICC= intercellular CO_2 concentration, STC= SPAD total chlorophyll, PH= plant height, LA= leaf area, NPT= number of productive tillers, FLA= flag leaf area, DW= dry weight, WFG= weight of filled grains, P= productivity, and AC= amylose content.

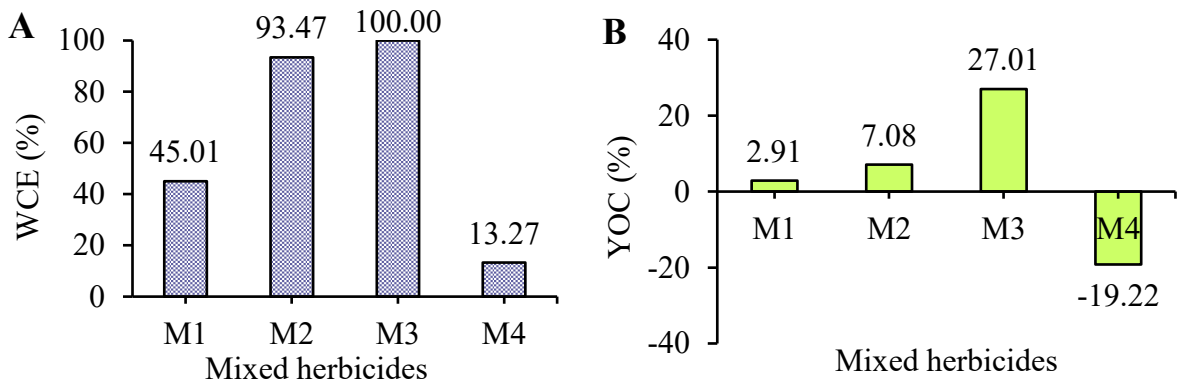


Figure 6. Weed control efficiency (A) and yield increase over control (B) of irrigated rice plants due to mixed herbicides. M1= cyhalofop-butyl+penoxsulam; M2= florpyrauxifen-benzyl+cyhalofop-butyl; M3= fenoxaprop-P-ethyl+ethoxysulfuron; and M4= bentazone+MCPA.

DISCUSSION

Experiment 1. Screening Testing

Based on the screening results, SB3 accession of barnyardgrass was classified as resistant to the recommended dose of bispyribac-sodium herbicide (103 g a.i.ha^{-1}) with a survival rate by 21.25% and the highest dry weight (Figure 2). This was possibly due to intensive irrigated rice farmers continuously spraying bispyribac-sodium herbicide over the last six growing seasons (Supplementary 1), resulting in strong selection pressure on these barnyardgrass and ultimately the growth of a resistant individual. These findings are supported by Vencill et al. (2012) that repeated and intensive use of a similar herbicide MoA creates selection pressure, leading to the evolution of resistance and difficulty in control. This evolution of a sensitive-to-resistant population results in mutations in the gene that inhibits the enzyme acetolactate synthase (ALS). Zhang et al. (2023) further reported that the primary mechanism of barnyardgrass resistance to ALS inhibitor herbicides is a substitution mutation from tryptophan to leucine at codon 574 in the ALS gene. This mutation alters the binding site, reducing the binding affinity for ALS inhibitor herbicides such as bispyribac-sodium, thereby reducing effectiveness. Cases of bispyribac-sodium resistant barnyardgrass in irrigated rice fields have been reported by previous studies. For example, Fuentes et al. (2025) identified 10 of 35 barnyardgrass accessions resistant to the bispyribac-sodium herbicide in irrigated rice fields, characterized by 100% survival and higher dry weight.

Experiment 2. Dose-Response Testing

The dose-response testing showed a decreasing pattern across all parameters of a barnyardgrass resistant-biotype (SB3) as the bispyribac-sodium dose increased to 16-fold the recommended rate. This indicates that the effectiveness of bispyribac-sodium was 100% at a

dose of 1,648 g a.i.ha^{-1} (Figure 3). Based on visual observations at this study site, the leaves of a barnyardgrass resistant-biotype (SB3) showed morphological abnormalities, including necrosis, yellowing, and browning at the stem base. These symptoms were clearly visible in some biotypes, particularly at 1 to 16-fold the recommended dose within a few days after application. The visual symptoms were confirmed by a decrease in SPAD total chlorophyll in the SB3 biotype at 7 DAA, with doses ranging from 1 to 16-fold (Figure 3B). Ultimately, the SB3 biotype at the 16-fold dose demonstrated 100% mortality rate at 21 DAA, while the susceptible population (MDN) had a 100% mortality rate at doses ranging from 1 to 16-fold. The relationship between morphological leaf-damage symptoms and the physiological response (SPAD total chlorophyll) of barnyardgrass due to bispyribac-sodium herbicide has been reported by several studies. Masum et al. (2019) found that a dose of 1,000 µM bispyribac-sodium herbicide significantly inhibited the growth of barnyardgrass weed, characterized by necrosis, leaf wilting, decreased total chlorophyll, and the presence of black spots on root nodes, as well as the absence of root hairs. Increasing bispyribac-sodium dose causes significant morphological and physiological damage to barnyardgrass, including reduced growth (Fuentes et al. 2025), chlorosis or yellowing of the leaves (Hamza et al. 2012), inhibition of the ALS enzyme (Zhang et al. 2023), as well as oxidative stress and damage to root cell structure (Fu et al. 2025). Alridiwirsah et al. (2023b) also found that the decline in total chlorophyll concentration of barnyardgrass offers a practical early marker for assessing the efficacy of herbicide applications.

Based on the dose-response testing, the SB3 biotype also showed a 5% survival rate at 8-fold the recommended dose. Therefore, this biotype remained capable of producing biomass and seed production. The capacity was due to the herbicide's frequent and intensive application, which led to the SB3 biotype evolving resistance up to 9.82-fold higher than the susceptible population (Table 3). This resistance index, as a benchmark indicates that the SB3 biotype is less effectively controlled by the bispyribac-sodium herbicide in irrigated rice fields. The resistance index of barnyardgrass resistant-biotype in irrigated rice fields has also been reported by previous studies. Duy et al. (2018) found that an 8-fold dose of the bispyribac-sodium herbicide could control a resistant biotype, achieving a mortality rate up to 97%. Fuentes et al. (2025) also found that the resistance index of barnyardgrass resistant-biotype increased from 3.1 to 16.7-fold compared to susceptible populations. Gobi et al. (2025) also demonstrated a 128-fold increase in barnyardgrass resistant-biotype to bispyribac-sodium herbicide compared to susceptible populations.

Experiment 3. Effect of Mixed Herbicides on Barnyardgrass Resistant-biotype Progenies

The P3 biotype progeny showed overall superior vegetative, physiological, histological, and yield characteristics compared to other biotype progenies (Tables 4-7 and Figure 4). Generally, resistant-biotype have higher detoxification enzyme activity than susceptible populations, including cytochrome P450 monooxygenases, glutathione S-transferases, and UDP-glycosyltransferases, as a non-target-site mechanism (Zhao et al. 2019; Wrzesińska-Krupa et al. 2023). The enzymes convert herbicides into non-toxic metabolites (Chen et al. 2025), thereby accelerating metabolism and reducing herbicide accumulation in leaf tissue, as indicated by increased photosynthesis and antioxidant capacities (Zhao et al. 2019). Consequently, the P3 biotype progeny had higher physiological characteristics than other biotype progenies. The physiological characteristics are closely related to leaf histology, such as stomatal density, mesophyll, and epidermis. The high stomatal density and leaf mesophyll tissue size in P3 biotype progeny at 7 DAA cause increased CO_2 diffusion to chloroplasts and have a positive impact on raising the transpiration rate, photosynthesis rate, stomatal conductance, and SPAD total chlorophyll. Additionally, the upper epidermis thickness can affect light penetration and gas exchange in the mesophyll, potentially influencing photosynthesis. These finding is supported by an increase in the size of histological tissues and physiological in the P3 biotype progeny, ranging from 8.03-19.47% and 9.07-267.19%, respectively compared to the P1 biotype progeny at 7 DAA (Table 6 and Figure 4). Furthermore, this resistant biotype recovered, as indicated by a 47.93% increase in SPAD total chlorophyll at 21 DAA (Figure 4). In the P3 biotype progeny, the increase in chlorophyll content caused a higher rate of carbon assimilation and biomass production, as indicated by a rise in vegetative traits of barnyardgrass ranging from 23.34-33.73%, dry weight 38.26%, flag leaves, and panicle length, as well as accelerate flowering (Tables 4 and 7). Gang et al. (2019) found that a thicker epidermal layer reduced light availability to the mesophyll, potentially affecting photosynthesis. Huang et al. (2022) also found that larger mesophyll cells and higher porosity increased CO_2 diffusion to chloroplasts, thereby enhancing photosynthesis. Eensalu et al. (2008) reported that higher stomatal density generally increased stomatal conductance, facilitating greater gas exchange in the mesophyll. Picoli et al. (2017) also found that the resistant-biotype showed higher recovery of photosynthesis activity at 28 DAA than susceptible populations.

The fenoxaprop-P-ethyl+ethoxysulfuron (M3) significantly affected the vegetative, physiological, and yield attributes of barnyardgrass resistant-biotype progenies up to 100%. Fenoxaprop-P-ethyl and ethoxysulfuron have different MoA but synergistically reduced the

mortality rate of barnyardgrass resistant-biotype progenies. Specifically, fenoxaprop-P-ethyl belongs to the aryloxyphenoxypropionate group, which inhibits the enzyme acetyl-CoA carboxylase (ACCase), thereby disrupting the production of essential fatty acids, impacting cell membrane integrity and the growth of *Echinochloa* weeds (Bakkali et al. 2007). Ethoxysulfuron targets a different metabolic pathway by inhibiting the enzyme acetolactate synthase (ALS), which is implicated in the synthesis of branched-chain amino acids (Zia-Ul-Haq et al. 2019). Consequently, cell division and weed growth are stopped. This dual inhibition of ACCase and ALS not only disrupts fatty acid and amino acid synthesis but also affects the histophysiological properties of the barnyardgrass, including lamina thickness, xylem vessel diameter, chlorophyll synthesis, and photosynthesis efficiency (Hamza et al., 2012; Akbulut et al., 2018). The histophysiological disturbances in the barnyardgrass biotype induced by herbicide mixtures were significant. The inhibition of the upper epidermis, mesophyll, lower epidermis, and stomatal density due to the fenoxaprop-P-ethyl+ethoxysulfuron (M3) up to 52.74% at 7 DAA compared to the control (Table 6). Similarly, physiological disturbances induced by the M3 herbicide were characterized by a decrease in transpiration rate, photosynthesis rate, and stomatal conductance at 7 DAA, as well as by SPAD total chlorophyll values up to 100% at 21 DAA (Figure 4). The M3 herbicide had a clear impact on barnyardgrass biotype mortality, as indicated by 100% weed control efficiency (Figure 6). These findings are supported by Hamza et al. (2012), who found that lamina thickness, xylem vessel diameter, chlorophyll a, b, and total of barnyardgrass resistant-biotype decreased when exposed to fenoxaprop-P-ethyl by 62.77; 30.56; 41.54; 19.58; and 33.44% compared to the unsprayed resistant-biotype. The decrease in lamina thickness due to the herbicide fenoxaprop-P-ethyl reflects a reduction in mesophyll cells, resulting from cell membrane damage, which inhibits cell division and enlargement. Akbulut et al. (2018); Zia-Ul-Haq et al. (2019) added that the combination of the herbicide fenoxaprop-P-ethyl and ethoxysulfuron synergistically controls barnyardgrass weeds by targeting oxidative stress, resulting in significant histophysiological inhibition. Chauhan and Abugho (2012) stated that fenoxaprop-P-ethyl+ethoxysulfuron is effective in controlling barnyardgrass up to 98%. Meher et al. (2024) also reported that the mixed herbicides of fenoxaprop-P-ethyl+ethoxysulfuron had the highest weed control efficiency by 86.49% in irrigated rice fields.

Experiment 3. Effect of Mixed Herbicides on Irrigated Rice

The results showed a decrease in agronomic and histophysiological attributes, as well as amylose content in irrigated rice grains grown simultaneously with the P3 biotype progeny (Tables 4-6, 8, and Figures 4-5). This was caused by physiological disturbances resulting from

suspected competition between the P3 biotype progeny and irrigated rice plants. Significant inhibition was observed in SPAD total chlorophyll, transpiration rate, and intracellular CO_2 content ranging from 4.05 to 30.53%, indicating that the P3 biotype progeny impaired photosynthesis and transpiration efficiency of irrigated rice plants (Figure 4E-H). This decrease in chlorophyll content indicates stress on the photosynthesis apparatus, exacerbated by reduced intracellular CO_2 availability for carbon fixation, thereby inhibiting energy and assimilate production. The disturbance was also reflected in the development of the upper epidermal tissue of irrigated rice leaves, which was inhibited by 16.31% (Table 6), ultimately affecting the integrity and function of the leaves as the primary photosynthesis organs. Although the effect was insignificant, the accumulation of these disturbances manifested as a tendency to inhibit vegetative growth up to 11.61% (Table 4), decreased productivity of 9.85%, and reduced amylose content in irrigated rice grains by 0.89% compared to the P1 biotype progeny (Table 8 and Figure 5). Therefore, P3 biotype progeny showed greater photosynthesis efficiency and adapted more quickly than irrigated rice plants when exposed to mixed herbicides in this study. This was due to the higher photosynthesis rate, transpiration rate, stomatal conductance, intracellular CO_2 content, and SPAD total chlorophyll in irrigated rice plants grown simultaneously with the P3 biotype progeny (Figure 4A-D). These findings are supported by Wu et al. (2023) that barnyardgrass resistant-biotype show increased photosynthesis-related protein content and higher chlorophyll content, indicating more adaptiveness to herbicide exposure than susceptible populations. According to Gao et al. (2019), in the herbicide-resistant barnyardgrass biotype, photosynthesis-related gene expression showed a low decrease in efficiency and recovered more quickly than in susceptible populations. Concenço et al. (2009) found that the transpiration rate of irrigated rice plants decreased by 14.29% when grown simultaneously with the barnyardgrass resistant-biotype compared to susceptible populations. The decrease in photosynthesis efficiency caused by the resistant-biotype reduced the productivity of irrigated rice plants. Tampubolon et al. (2025) reported a decrease in irrigated rice productivity ranging from 5.13 to 83.59% when grown simultaneously with barnyardgrass that was intensively exposed to the herbicides. Similarly, the decrease in amylose content in irrigated rice grains caused by the P3 biotype progeny is due to the resulting low photosynthesis rate. This decrease in photosynthesis rate is linearly correlated with a reduction in stomatal conductance. Ono et al. (2013) also reported that reduced stomatal conductance can decrease the photosynthesis rate, thereby inhibiting grain filling and starch synthesis in irrigated rice.

The effects of mixed herbicides varied on the agronomic and histophysiological attributes of irrigated rice plants. All mixed herbicides significantly reduced SPAD total

chlorophyll in irrigated rice for several DAA, after which it gradually recovered by 7 DAA, particularly for cyhalofop-butyl+penoxsulam (M1) and fenoxaprop-P-ethyl+ethoxysulfuron (M3). However, the M3 herbicide significantly increased the photosynthesis rate of irrigated rice plants. It proved superior in increasing the number of productive tillers, flag leaf area, dry weight, panicle length, number and weight of filled grains, as well as productivity ranging from 6.20 to 61.21% compared to the control, and accelerated flowering to 69.56 days. Additionally, M3 herbicide significantly increased the amylose content of irrigated rice grains, while M1 and M4 herbicides caused a decrease. This indicates that fenoxaprop-P-ethyl+ethoxysulfuron (M3) is selective and safe with respect to the agronomic attributes of irrigated rice plants. The result is possibly due to the lower mobility of the herbicide active ingredient in irrigated rice plant tissue compared to the barnyardgrass resistant-biotype progeny during spraying. Additionally, the fenoxaprop-P-ethyl is more readily degradable in irrigated rice plant tissue. Lu et al. (2022) found that the mobility of the herbicide fenoxaprop-P-ethyl in the phloem tissue of barnyardgrass was 4.04-fold higher compared to irrigated rice plants. Guo et al. (2008) and Wu et al. (2015) reported that the herbicide fenoxaprop-P-ethyl decomposed rapidly in irrigated rice plant tissue with half-lives of 2.14 days and 1.26-1.41 days, respectively. Furthermore, Kaur et al. (2025) mentioned that the herbicide ethoxysulfuron had a hazard quotient index of 6.57×10^{-4} with a decomposition half-life of 9.90-12.19 days, making it safe for the growth of irrigated rice plants. The selectivity of fenoxaprop-P-ethyl, characterized by low translocation and rapid degradation in the phloem tissue of irrigated rice plants, and the low hazard index of ethoxysulfuron confirms the synergistic efficacy of their combination in suppressing 100% mortality of barnyardgrass resistant-biotype (Figure 6A). The mortality of this biotype progeny eliminates competition for light intensity and nutrients, thereby increasing irrigated rice biomass and production. This is confirmed by Table 9, showing that irrigated rice productivity is significantly positively correlated with the upper epidermis tissue by 0.348. The correlation occurs because the upper epidermis presumably increases intercellular CO_2 concentration, which ultimately stimulates photosynthesis rate and promotes higher biomass and yield. The effectiveness of fenoxaprop-P-ethyl+ethoxysulfuron has been reported to increase irrigated rice yields by 226-273% compared to the control (Zia-Ul-Haq et al. 2019). Similarly, the amylose content of irrigated rice grains in this study was influenced by several enzymatic metabolic pathways that were indirectly associated with amylose synthesis following application of fenoxaprop-P-ethyl+ethoxysulfuron. Zhang et al. (2020) found that the impact of fenoxaprop-P-ethyl+ethoxysulfuron on plant biochemistry, such as antioxidant enzymes and lipid peroxidation, is related to the accumulation of precursor molecules, including glucose-1-

phosphate with the potential to increase the activity of the granule-bound starch synthase enzyme in amylose synthesis. Seo et al. (2015) reported an increase in the amylose content of irrigated rice grains due to the application of mefenacet+pyrazosulfuron-ethyl and bentazone+cyhalofop-butyl by 1.52 and 3.07%, respectively. For additional information, Tandon (2017); Kaur et al. (2025) demonstrated that residues of the fenoxaprop-P-ethyl and ethoxysulfuron herbicides in irrigated rice grains were below the safe threshold. This indicates that fenoxaprop-P-ethyl+ethoxysulfuron is safe for irrigated rice quality, as evidenced by increased amylose content.

Principal findings of this study, the herbicides fenoxaprop-P-ethyl+ethoxysulfuron achieved 100% control efficiency for the bispyribac-sodium resistant of barnyardgrass biotype and resulted in a 27.01% increase in irrigated rice productivity (Figure 6). These results demonstrate that resistant weed management can be achieved through herbicide MoA rotation. Fenoxaprop-P-ethyl+ethoxysulfuron, as an active ingredient, is recommended to farmers to address failures in barnyardgrass control in irrigated rice fields. To support this recommendation, the government and agricultural extension workers should facilitate outreach and training to endorse investigation-based solutions to farmers and provide access to mixed herbicides to prevent the spread of weed resistance in irrigated rice fields.

CONCLUSION

This study confirmed that barnyardgrass with SB3-accession from irrigated rice fields showed 9.82-fold resistance to bispyribac-sodium compared to the susceptible. This indicates that the SB3-accession will be difficult to control when farmers continue using this herbicide. Management of barnyardgrass resistant-biotype requires a MoA rotation approach with mixed herbicides. Fenoxaprop-P-ethyl+ethoxysulfuron was identified as the most effective herbicide mixture in histophysiological inhibition of barnyardgrass across all resistant-biotype progenies (low, labeled, and high-dose), resulting in 100% mortality. This was followed by florpyrauxifen-benzyl+cyhalofop-butyl with a control efficiency of 93.47%. Additionally, fenoxaprop-P-ethyl+ethoxysulfuron significantly improved yield attributes by 6.20-61.21% and rice productivity at 27.01%. It was also classified as safe, based on a 31.50% increase in grain amylose content relative to the control. These comprehensive results show that the management of barnyardgrass resistant-biotype to bispyribac-sodium can be achieved through a combination of different MoA and may be a useful solution for farmers, agronomists, and policymakers.

Conflicts of interest

The authors report none conflicts of interest regarding this study.

Authors' Contributions

Collecting barnyardgrass seeds from irrigated rice (Koko Tampubolon, Alridiwirsah); Screening tests of barnyardgrass accessions (Koko Tampubolon, Alridiwirsah, Mohammad Basyuni); Dose-response tests of resistant-biotypes (Koko Tampubolon, Alridiwirsah, Novilda Elizabeth Mustamu, Posma Mangasi Pintaria Marbun); Management testing of resistant-biotypes progenies using mixed herbicides (Koko Tampubolon, Alridiwirsah, Mohammad Basyuni, Posma Mangasi Pintaria Marbun, Risnawati); Data collection (Risnawati, Sanggam Dera Rosa Tampubolon, Zuziana Susanti); Data processing (Koko Tampubolon, Posma Mangasi Pintaria Marbun, Novilda Elizabeth Mustamu, Risnawati); Amylose analysis in rice grains (Sanggam Dera Rosa Tampubolon, Zuziana Susanti, Koko Tampubolon); Writing, revising, and publication (all authors).

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Supplementary 1. History of weed control practices employed by farmers in irrigated rice at Sei Bamban Subdistrict, Serdang Bedagai District, North Sumatra, Indonesia, during the period 2018-2023

Accession code	Farmers (F)	Years						Selected accession
		2018	2019	2020	2021	2022	2023	
MDN	F1	I	I	I	I	F	I	-
	F2	I	F	I	I	I	I	-
	F3	I	I	I	I	F	F	-
	F4	I	I	I	I	I	I	MDN
	F5	I	I	F	F	F	F	-
SB	F1	E	A	A	B	G	A	-
	F2	F	A	A	A	A	H	SB1
	F3	E	A	A	D	H	B	-
	F4	F	E	A	E	B	B	-
	F5	F	E	E	E	A	H	-
	F6	F	F	A	G	B	G	-
	F7	E	A	A	A	G	A	SB2
	F8	E	A	A	C	B	A	-
	F9	F	F	F	D	B	B	-
	F10	E	F	F	H	G	H	-
	F11	F	A	A	E	B	H	-
	F12	F	A	A	B	C	G	-
	F13	E	A	D	G	B	A	-
	F14	E	A	A	G	A	H	SB3
	F15	E	A	C	B	H	A	-
	F16	E	E	F	A	B	G	-
	F17	F	F	E	B	B	B	-
	F18	F	A	A	G	H	A	SB4
	F19	E	E	D	E	G	A	-
	F20	F	A	C	C	B	A	-
	F21	E	F	A	B	A	B	-
	F22	E	E	C	G	A	H	-
	F23	F	E	E	D	H	H	-
	F24	E	E	F	A	A	A	-
	F25	E	A	A	H	A	G	SB5

Note: MDN and SB refer to weed accessions collected from Medan and Sei Bamban. Weed control treatments; A= bispyribac-sodium; B= fenoxaprop-P-ethyl; C= profoxydim; D= cyhalofop-butyl+ethoxysulfuron; E= metsulfuron-methyl+chlorimuron-ethyl+2,4-D sodium; F= 2,4-D dimethylamine; G= bensulfuron-methyl+bispyribac-sodium; H= bispyribac-sodium+pyrazosulfuron; and I= manual weeding.