

Agro-histological performances and seed production of barnyardgrass (*Echinochloa crus-galli*) to 2,4-D dimethylamine in lowland rice fields

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Abstract: Background: Herbicide exposure affects the agro-histological characteristics and seed production of barnyardgrass in lowland rice fields. **Objective:** This study aimed to assess the impact of the 2,4-D dimethylamine on the agro-histological and seed production of barnyardgrass and its effects on lowland rice plants. **Methods:** Barnyardgrass seeds were collected from lowland rice fields in nine sub-districts of Serdang Bedagai District, Indonesia. The study was conducted in farmer fields in Medan Selayang, Indonesia, from August to December 2022. The study used a randomized block design with a single factor, 40

populations of barnyardgrass sprayed the 2,4-D dimethylamine at 1.5 fold of the recommended dose (1,080 g ai ha⁻¹) with three replications. **Results:** The increase in tissue size of the upper epidermis, mesophyll, and lower epidermis can be used as an indicator of the effect of higher doses of 2,4-D dimethylamine. The herbicide 2,4-D dimethylamine at a dose of 1.5 fold caused an increase in the seeds produced of barnyardgrass biotypes up to 8.20 fold and lowland rice yield until 5.98 tons ha⁻¹. **Conclusions:** The herbicide remained safe for the growth and yield of lowland rice and enlarge the agro-histological tissues of barnyardgrass.

Keywords: 2,4-D Dimethylamine; Agronomy; Barnyardgrass; Distribution; Histological

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1. Introduction

Barnyardgrass (*Echinochloa crus-galli*) is a significant weed in lowland rice fields, causing substantial yield losses. The presence of this weed at a density of 25 populations m⁻² can lead to 50% yield loss in lowland rice (Chin, 2001) and produce up to 39,000 seeds per plant (Bagavathiannan et al., 2012). Furthermore, Zhang et al. (2017) reported that barnyardgrass has the potential to inhibit various growth aspects, such as photosynthesis rate, root weight, root oxidation activity, and biomass accumulation, ultimately reducing grain production of lowland rice ranging from 12.7 to 42.6%. Barnyardgrass control is crucial in the central rice-producing regions in Indonesia, especially in North Sumatra Province. The data from the Statistics of Sumatera Utara (2022) showed that Serdang Bedagai District, encompassing an area of 48,121.62 ha, held the distinction of being the second-largest harvested area for rice crops in North Sumatra, with a productivity of 5.58 tons ha⁻¹ in 2021. However, this productivity has indicated a decline from 6.09 tons ha⁻¹ in 2020. The presence of major weed populations such as barnyardgrass could potentially be a contributing factor to the reduction in lowland rice productivity in Serdang Bedagai District, Indonesia.

Based on interviews with several lowland rice farmers in Serdang Bedagai, *E. crus-galli* weeds were frequently sprayed using the herbicide 2,4-D dimethylamine. 2,4-D represents one of the earliest synthetic auxin herbicides, widely recognized and used for controlling both annual and perennial weeds (Peterson, 1967). Mithila et al. (2011) and Peterson et al. (2016), Its popularity stems from several advantageous features, including low cost, selective targeting, and broad-spectrum weed control. 2,4-D dimethylamine herbicide at the recommended dose or 720 g ai ha⁻¹ effectively controlled 67.37% of the barnyardgrass dry weight in lowland rice fields (Alridiwersah et al. 2020a). Despite its apparent effectiveness, the use of auxin-type herbicides, such as 2,4-D, has raised concerns due to their potential adverse effects on plant growth. Symptoms induced by these herbicides mirror those triggered by high doses of natural auxin hormones, such as indole-3-acetic acid (IAA). At low doses, these herbicides promote plant growth, but at higher doses, they cause excessive growth, resulting in cupping and stunting of leaves, stunting and twisting of stems, brittleness, and abnormal growth (Grossmann, 2010).

Agro-histological changes in lowland rice plants and *E. crus-galli* with exposure to 2,4-D dimethylamine herbicide at a recommended dose have been reported. Alridiwersah et al. (2022) found that the histological characteristics of *E. crus-galli* were changed due to exposure to 2,4-D dimethylamine at a recommended dose or 720 g ai ha⁻¹ compared to untreated. Weed control in primary plants with a recommended dose has been reported by Alridiwersah et al. (2020b); Tampubolon et al. (2020; 2024); Önemli and Tetik, (2023); and Süer et al. (2024). However, changes in the agro-histological features of plants when the dose of 2,4-D dimethylamine has been increased from a recommended dose. Agro-histological studies allow the best evaluation of injuries caused by higher doses of the 2,4-D dimethylamine, as they provide important information on structural changes in morphology and anatomical tissues. This study aimed to assess the impact of the 2,4-D dimethylamine on the agro-histological and seed produced of barnyardgrass and lowland rice plants.

2. Material and Methods

2.1 Seed Source of Barnyardgrass Weed

Barnyardgrass seeds were collected from lowland rice fields, encompassing a total of 40 populations from nine sub-districts in Serdang Bedagai District, as indicated by Alridiwersah et al. (2023a) in Table 1. Based on interviews with farmers, it was evident that this particular weed was consistently managed using the 2,4-D dimethylamine. To establish a comparative reference, a population of barnyardgrass (denoted as ECG-0) was collected from Medan Selayang, Indonesia. The collection location of this weed from lowland rice fields in Medan and each district in Serdang Bedagai were visually represented in Figure 1. To ensure consistency and reliability in the seed collection process, specific criteria were employed for selecting barnyardgrass seeds. Physiologically mature seeds were targeted, characterized by their tendency to shatter upon handling. These mature seeds were placed in brown envelopes, labeled for identification purposes, and subsequently dried for 2 days.

2.2 Study Site and Establishing Growing Media

This study was conducted in farmer fields located in Padang Bulan, Medan Selayang, Indonesia, at geographic coordinates of 3°33.764' N and 98°38.895' E at an elevation of 15 m A.S.L. from August to December 2022. The soil of lowland rice was collected and carefully transferred into buckets. The buckets had a capacity filled to three-quarters, with an upper diameter, lower diameter, and height were 37.5; 28; and 15 cm, as indicated in Figure 2. The soil was then subjected to flooded conditions for a week.

Table 1 - Barnyardgrass seeds were collected at lowland rice fields in nine sub-districts from Serdang Bedagai, Indonesia

Sub-districts	Number of populations	Population code
Sei Bamban	5	ECG-1, ECG-2, ECG-3, ECG-4, ECG-5
Sei Rampah	5	ECG-6, ECG-7, ECG-8, ECG-9, ECG-10
Tebing Tinggi	3	ECG-11, ECG-12, ECG-13
Perbaungan	5	ECG-14, ECG-15, ECG-16, ECG-17, ECG-18
Teluk Mengkudu	5	ECG-19, ECG-20, ECG-21, ECG-22, ECG-23
Pegajahan	4	ECG-24, ECG-25, ECG-26, ECG-27
Pantai Cermin	3	ECG-28, ECG-29, ECG-30
Tanjung Beringin	5	ECG-31, ECG-32, ECG-33, ECG-34, ECG-35
Bandar Khalipah	5	ECG-36, ECG-37, ECG-38, ECG-39, ECG-40
Total	40	

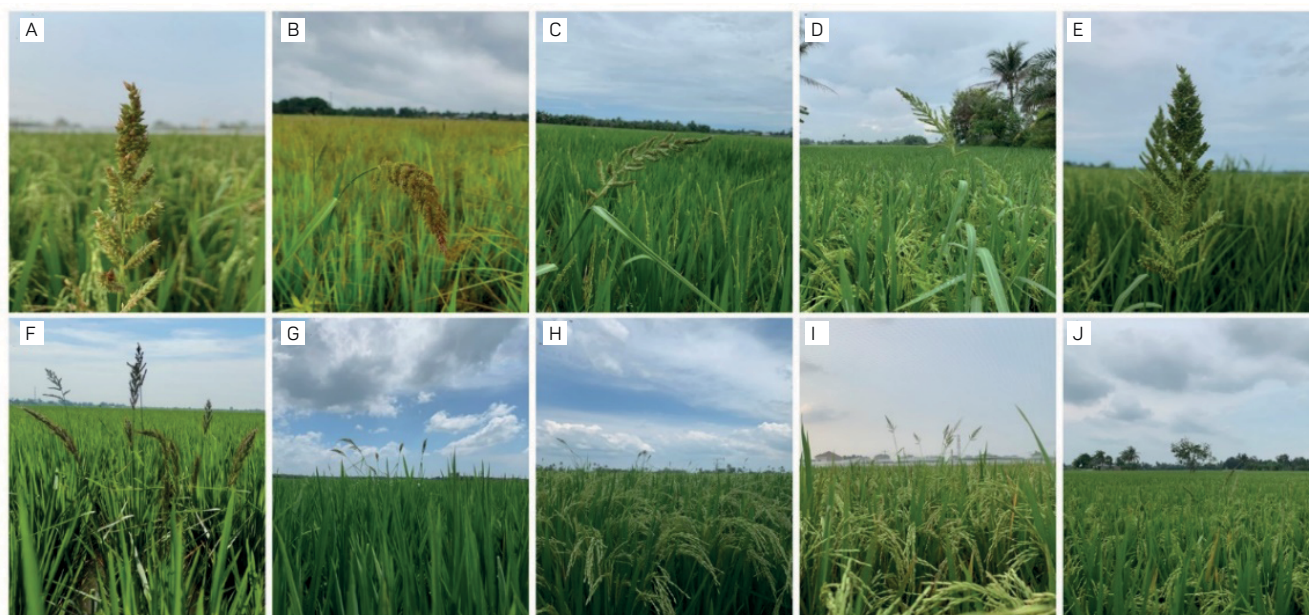
Note: ECG: *Echinochloa crus-galli*

2.3 Germination until Transplanting of Lowland Rice and Barnyardgrass

For this study, the Ciherang variety of lowland rice seeds was selected as the primary plant with an average productivity of 6.3 tons ha⁻¹ (Ministry of Agriculture, 2015). These rice seeds were germinated and grown until 3-5 leaves, indicating an appropriate stage for transplanting. Subsequently, one seedling of lowland rice was carefully transplanted into each bucket, ensuring uniformity and consistency in the experimental setup. Barnyardgrass seeds were also germinated and grown until 2-3 leaves, deemed suitable for transplanting. To create representative populations, 20 seedlings of barnyardgrass were transplanted into each bucket, as shown in Figure 2.

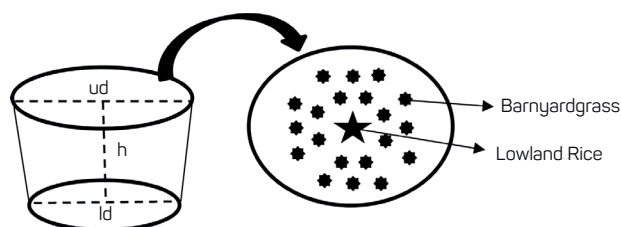
2.4 Study Design and Herbicide Application

Following the planting phase, a randomized block design was employed, with the barnyardgrass population serving as the single factor under investigation with three replications. The buckets were arranged based on the spacing of the lowland rice plants, namely 20 cm × 20 cm. To ensure accurate and precise herbicide application, the spraying calibration was calculated, resulting in a spraying rate of 289.81 L ha⁻¹. The 2,4-D dimethylamine was sprayed at a dose of 1,080 g ai ha⁻¹ when barnyardgrass reached the developmental stage with 4-5 leaves. The spraying was performed at 10:10 AM local time, with temperatures, humidity, and air pressure recorded as 29.7°C, 66%, and 1,010 hPa, respectively. The lowland rice and barnyardgrass was fertilized with NPK at a dose of 440 kg ha⁻¹, which was



Medan City (A) and nine sub-districts in Serdang Bedagai (B= Sei Bamban, C= Sei Rampah, D= Tebing Tinggi, E= Perbaungan, F= Teluk Mengkudu, G= Pega-jahan, H= Pantai Cermin, I= Tanjung Beringin, J= Bandar Khalipah).

Figure 1 - Barnyardgrass in lowland rice fields.



ud: upper diameter, Id: lower diameter, h: height

Figure 2 - The bucket size and planting placement of barnyardgrass seedlings with lowland rice

equivalent to 1.76 g per plant, and it was applied three times, including before planting, at 25 days after planting (DAP), and at 50 DAP. Deltamethrin 25 g L⁻¹ (insecticide) and mancozeb 80% (fungicide) were applied following recommended guidelines.

2.5 Parameters and Data Analysis

The assessment of herbicide effects involved measuring various parameters for lowland rice plants and barnyardgrass. Data collected for lowland rice plants included plant height (cm), number of leaves and tillers, leaf area (cm²), length and width of the flag leaf (cm), flowering age (days), number of productive tillers, panicle length (cm), number of grains, fresh weight (g), dry weight (g), grains weight (g), 1,000-seeds weight (g), yield (tons ha⁻¹), and yield decline (%). Certain measurements were taken before herbicide spraying. The plant height, number of leaves and tillers, as well as leaf area of rice plants were

measured at 3 Weeks After Spraying (WAS), while other data were measured at the end of this study (14 WAS) when the harvest criteria were achieved and the grains exhibited >80% yellowing. As indicated by Gomez and Gomez (1976), the calculation of leaf area (A) involved measuring the length (L) and width (W) of the second fully opened leaf from the tip, using the formula $A = LWc$ ($c=0.76$), and leaf width was measured using a digital caliper. Upon completion of the study, the basal part of the lowland rice plant stem was cut, weighed to determine the fresh weight, and subsequently oven-dried at 80°C for 48 h (Scientific Engineering Response and Analytical Services, 1994) to obtain the dry weight. The data of lowland rice characteristics were analyzed using Pearson correlation. Measurement of yield and yield decline using equations 1-2.

$$\text{Yield (tons ha}^{-1}\text{)} = \frac{\text{Grains weight}}{1,000} \times \frac{\text{land area per ha}}{\text{plant spacing area}} \quad (1)$$

$$\text{Yield decline (\%)} = \frac{\frac{\text{Average yield of descriptions-}}{\text{Average yield of treatment}}}{\frac{\text{Average yield of}}{\text{descriptions}}} \times 100\% \quad (2)$$

The measurements of barnyardgrass data encompassed various parameters, including the number of tillers, plant height (cm), length and width of leaves (cm), flowering age (days), seed maturity age (days), length and width of flag leaf (cm), panicle length (cm), number of seeds, fresh weight (g), dry weight (g), and leaf histological tissues (upper epidermis, mesophyll, lower epidermis, stomatal density). Certain measurements were taken

before herbicide spraying. The number of tillers, plant height, and length and width of leaves were measured at 3 WAS. Leaf histological data were collected at 1 WAS, and all other data were measured at the end of seed harvest (11 WAS). Following seed harvest completion, the basal part of the barnyardgrass stem was cut and weighed to obtain the fresh weight, and subsequently oven-dried at 65°C for 72 h to determine the dry weight. The measurements of the upper epidermis, mesophyll, and lower epidermis tissue sizes (μm) in barnyardgrass leaves were performed by obtaining the first leaf from the tip using transverse paraffin sections (Johansen, 1940). Images were captured through the application of Axiovision 4.8 at a magnification of 10×10 . Stomatal density ($\text{n}/\mu\text{m}$) was measured by applying clear nail polish on the lower leaf surface, letting it dry for a few minutes, and then sticking clear tape on it. The dried tape was pulled off and placed on a slide for stomatal density counting using a microscope, and leaf width was measured using a caliper. Subsequently, the data on lowland rice are only processed by standard errors and analyzes Pearson correlation between agronomic characteristics with productivity. Meanwhile, the data of barnyardgrass were analyzed by employing one-way ANOVA and followed by DMRT at a significance level of 5% using IBM SPSS v.20.

3. Results

3.1 Agronomic Performances of Lowland Rice

The growth and yield characteristics of lowland rice plants due to the 2,4-D dimethylamine herbicide were presented in Table 2. The plant height and number of leaves in lowland rice plants were not affected by exposure to the 2,4-D dimethylamine herbicide. However, hindrance in the number of tillers and leaf area growth was observed after 3 weeks of 2,4-D dimethylamine herbicide exposure. Among the 41 lowland rice plants, 23 plants (ECG 1-3, 6, 7, 10-12, 14, 15, 17-20, 23, 26, 28-30, 32, 35, 36, and 39) experienced a decrease in the number of tillers at 3 WAS. The highest number of tillers inhibition was found in ECG-10 at 26.59%, while the lowest (1.80%) was observed in ECG-15. Similarly, 13 of 41 lowland rice plants, namely ECG-1, 2, 4, 12, 18, 20, 26, 28, 29, 32, 33, 35, and 39 experienced a decrease in leaf area. The highest leaf area inhibition was 25.82% in ECG-26, while the lowest (0.14%) was found in ECG-2. Several agronomic characteristics of lowland rice plants, including the length and width of the flag leaf, flowering age, number of productive tillers, panicle length, number of grains, fresh weight, dry weight, grains weight, 1,000-seeds weight, and yield exhibited significant variation, ranging from 19.77-32.37 cm; 0.99-1.29 cm; 27.00-50.67 days; 8.00-18.33 tillers; 19.73-26.03 cm; 217.67-984.67 grains; 55.41-182.32 g; 14.32-69.42 g; 4.14-23.91 g; 12.88-28.08 g; and 1.03-5.98 tons ha^{-1} , respectively. Based on the description of lowland rice

variety in this study, it was found that yield decline ranged from 5.13-83.59%. The correlation analysis showed that the lowland rice yield exposed to the 2,4-D dimethylamine herbicide significantly correlated with plant height, length and width of the flag leaf, number of productive tillers, fresh and dry weight, flowering age, panicle length, number of grains, and 1,000-seeds weight (Table 3).

3.2 Agro-histological Performances of Barnyardgrass

The application of 2,4-D dimethylamine herbicide at a dose of 1,080 g ai ha^{-1} significantly affected the agronomic and leaf histological performances of the barnyardgrass, except for leaf length (Table 4 and Figure 3). Furthermore, the herbicide demonstrated reduced efficacy in suppressing the growth and leaf histological tissue of the barnyardgrass biotypes while being effective in suppressing the comparison population (ECG-0). The ECG-12 exhibited earlier flowering and seed maturity ages, occurring at 27.00 and 38.33 days, respectively. Generally, the characteristics of the barnyardgrass biotypes due to the 2,4-D dimethylamine herbicide ranged as, the number of tillers (8.33 to 38.00), length and width of leaves (23.87 to 37.43 cm and 0.46 to 1.01 cm), plant height (38.12 to 64.00 cm), length and width of flag leaf (9.00 to 25.90 cm and 0.83 to 1.35 cm), panicle length (10.03 to 15.97 cm), and fresh weight (93.11 to 340.09 g), and dry weight (21.68 to 129.18 g). Similarly, the leaf histological characteristics of the barnyardgrass biotypes including the upper epidermis, mesophyll, lower epidermis, and stomatal density, also ranged from 13.91 to 42.15 μm , 41.52 to 151.32 μm , 11.35 to 29.58 μm , and 19.79 to 59.46 $\text{n}/\mu\text{m}$, respectively. The application of herbicide 2,4-D dimethylamine at a dose of 1,080 g ai ha^{-1} significantly increased the seeds produced of barnyardgrass biotypes compared to the comparison population (Figure 4). The seeds produced by the barnyardgrass biotypes ranged from 1298.00 to 9457.33 seeds or 1.13 to 8.20 times compared to the comparison population. It indicates that the use of these herbicide doses can enhance seed distribution in the soil and is considered disadvantageous to the yield of lowland rice in the future.

Table 5 revealed that the seeds produced of barnyardgrass was significantly and positively correlated with the number of tillers and flag leaf length (0.413^{***} and 0.310^{*}). However, the characteristics of the weed height, leaves length, mesophyll tissue, stomatal density, and biomass were positively correlated with the seeds produced of barnyardgrass.

4. Discussion

The application of the 2,4-D dimethylamine at a dose of 1,080 g ai ha^{-1} led to the inhibition number of tillers and leaf area in lowland rice plants at 3 WAS (Table 2). The highest observed inhibitions were 26.59% for the number

Table 2 - Agronomic characteristics of lowland rice plants after exposure to 2,4-D dimethylamine herbicide										
Lowland Rice	Plant height (cm)		Number of leaves		Number of tillers		Leaf area (cm ²)		Flag leaf (cm)	
	Before spraying	3WAS	Before spraying	3WAS	Before spraying	3WAS	Before spraying	3WAS	Length	Width
ECG-0	44.73±0.39	56.17±0.10	46.00±1.08	129.67±1.01	22.00±0.56	24.00±0.39	19.63±0.28	23.60±0.06	27.37±0.32	1.27±0.00
ECG-1	46.33±0.12	56.13±0.24	46.33±0.44	98.67±0.28	19.00±0.39	18.33±0.23	21.30±0.19	19.29±0.17	23.00±0.49	1.17±0.01
ECG-2	50.53±0.15	59.57±0.55	47.67±0.75	95.00±1.31	22.00±0.31	17.67±0.19	21.82±0.33	21.79±0.28	32.37±1.00	0.99±0.01
ECG-3	41.53±0.63	52.80±0.63	32.67±1.43	77.33±3.99	14.67±0.81	12.33±0.85	16.06±0.50	19.32±0.51	22.83±0.04	1.09±0.01
ECG-4	48.43±0.43	54.33±0.41	45.00±0.33	95.33±1.34	19.33±0.05	19.33±0.14	22.10±0.25	17.59±0.32	26.23±0.78	1.23±0.00
ECG-5	46.77±0.24	52.00±0.08	38.33±0.91	85.33±1.73	16.67±0.19	17.67±0.34	20.37±0.10	21.02±0.62	27.30±0.46	1.19±0.00
ECG-6	46.87±0.08	55.83±0.46	43.67±0.38	107.33±1.06	21.00±0.09	18.67±0.29	18.06±0.08	18.51±0.19	27.10±0.56	1.17±0.01
ECG-7	42.73±0.11	52.17±0.05	42.00±0.24	94.00±1.18	16.67±0.23	14.00±0.09	18.30±0.50	18.72±0.23	20.67±0.10	1.08±0.01
ECG-8	48.03±0.49	55.50±0.33	41.33±0.82	112.67±0.50	20.33±0.41	21.00±0.24	19.10±0.37	21.22±0.23	27.37±0.10	1.19±0.01
ECG-9	45.57±0.35	63.77±0.13	46.33±0.61	117.67±1.86	15.00±0.50	32.67±0.32	23.47±0.59	31.11±0.71	26.23±0.66	1.13±0.00
ECG-10	48.43±0.26	56.37±0.15	50.00±1.36	112.33±1.49	26.33±0.38	19.33±0.29	21.25±0.42	24.12±0.43	25.50±0.23	1.23±0.00
ECG-11	46.23±0.42	61.97±1.37	43.33±0.29	98.33±0.90	19.67±0.23	18.33±0.05	17.74±0.22	18.18±0.22	23.97±0.20	1.12±0.01
ECG-12	44.73±0.20	51.87±0.25	44.33±0.50	92.33±0.99	19.00±0.24	14.67±0.21	17.38±0.39	15.34±0.16	26.10±0.37	1.16±0.00
ECG-13	42.60±0.19	58.63±0.10	34.67±0.65	101.67±2.09	14.00±0.33	14.67±0.41	16.64±0.31	30.30±0.24	29.33±0.16	1.26±0.01
ECG-14	42.57±0.10	49.60±0.06	40.67±0.65	88.67±0.42	20.00±0.33	15.33±0.34	14.98±0.04	15.90±0.28	26.00±0.15	1.07±0.00
ECG-15	50.60±0.20	53.83±0.10	46.67±0.32	107.00±1.28	18.33±0.10	18.00±0.24	21.25±0.25	21.39±0.21	24.10±0.17	1.12±0.00
ECG-16	46.37±0.22	60.13±0.26	35.00±0.39	89.67±1.05	12.00±0.24	26.67±0.63	25.67±0.22	36.90±0.34	27.07±0.40	1.19±0.01
ECG-17	48.23±0.30	51.83±0.21	44.00±0.47	87.00±1.56	17.33±0.19	16.67±0.14	18.71±0.08	23.15±0.44	29.87±0.33	1.17±0.01
ECG-18	49.70±0.41	55.13±0.21	48.67±0.64	103.67±2.05	25.33±0.64	19.00±0.16	20.62±0.19	17.41±0.32	22.73±0.74	1.16±0.00
ECG-19	48.50±0.27	54.50±0.38	44.67±1.10	77.67±1.24	18.33±0.32	14.67±0.10	19.04±0.46	23.84±0.54	25.80±0.62	1.18±0.01
ECG-20	48.60±0.63	51.83±0.56	51.33±0.23	121.33±1.34	21.67±0.19	20.00±0.24	19.49±0.48	18.74±0.18	22.40±0.32	1.16±0.00
ECG-21	45.07±0.18	52.40±0.31	50.00±0.74	125.00±0.56	21.33±0.14	21.33±0.14	14.92±0.15	19.14±0.28	24.87±0.49	1.21±0.00
ECG-22	49.87±0.05	58.10±0.19	52.33±0.32	125.33±1.13	21.67±0.28	22.67±0.14	19.42±0.17	22.85±0.46	26.00±0.57	1.26±0.01
ECG-23	43.27±0.42	50.87±0.24	35.33±0.57	79.33±1.59	17.33±0.46	15.67±0.21	17.28±0.11	18.30±0.05	25.70±0.22	1.13±0.01
ECG-24	48.67±0.17	64.83±0.28	54.33±0.21	136.67±0.50	20.00±0.09	32.00±0.78	27.53±0.32	35.71±0.48	25.93±0.25	1.24±0.00
ECG-25	52.47±0.51	63.93±0.73	47.33±0.36	125.00±0.86	16.33±0.21	28.33±0.68	30.13±0.19	38.80±0.39	19.77±0.41	1.12±0.00
ECG-26	46.70±0.27	51.80±0.30	40.00±0.68	84.33±1.05	17.33±0.14	14.67±0.26	21.77±0.22	16.15±0.26	24.83±0.23	1.18±0.00
ECG-27	48.37±0.50	63.50±0.71	47.33±0.57	125.00±0.65	18.33±0.46	33.33±1.03	28.02±0.54	34.95±0.62	27.17±0.33	1.18±0.01
ECG-28	44.07±0.53	49.93±0.35	36.33±0.79	77.67±2.40	18.67±0.26	14.33±0.41	17.68±0.35	16.28±0.24	21.80±0.19	1.05±0.01
ECG-29	45.33±0.24	50.43±0.27	37.67±0.82	94.67±2.03	18.33±0.34	15.00±0.39	19.50±0.12	17.75±0.32	24.60±0.33	1.18±0.01
ECG-30	49.50±0.14	58.00±0.20	48.67±0.41	114.67±1.08	23.67±0.14	20.67±0.14	21.03±0.22	22.14±0.07	24.20±0.13	1.24±0.00
ECG-31	47.93±0.19	58.57±0.27	48.67±0.73	130.33±3.00	20.00±0.39	22.00±0.33	18.17±0.40	23.05±0.43	24.50±0.93	1.14±0.01
ECG-32	45.80±0.51	50.83±0.99	47.33±0.64	115.67±2.18	22.00±0.33	19.33±0.36	19.71±0.62	18.59±0.84	21.67±0.62	1.09±0.03
ECG-33	46.90±0.18	57.93±0.36	44.33±0.82	112.00±1.86	21.67±0.10	21.67±0.32	20.56±0.15	18.06±0.11	27.73±0.28	1.21±0.01
ECG-34	45.57±0.23	56.60±0.20	47.33±0.53	112.33±2.30	22.67±0.54	22.00±0.86	19.38±0.10	21.89±0.30	26.47±0.52	1.27±0.00
ECG-35	44.63±0.54	51.47±0.16	43.00±0.79	91.00±1.53	19.00±0.47	18.33±0.41	19.65±0.37	19.08±0.17	22.00±0.24	1.21±0.00
ECG-36	43.20±0.22	55.90±0.12	31.33±0.38	83.33±0.51	17.00±0.18	13.67±0.10	17.91±0.17	27.59±0.44	28.20±0.48	1.16±0.00
ECG-37	47.47±0.31	56.53±0.42	35.33±0.65	114.33±1.15	18.67±0.14	19.33±0.14	19.20±0.38	23.64±0.32	21.90±0.31	1.24±0.00
ECG-38	43.33±0.39	60.33±0.19	31.67±0.60	101.33±0.54	14.33±0.38	23.00±0.00	15.73±0.09	26.99±0.22	26.97±0.42	1.17±0.01
ECG-39	49.07±0.56	55.20±0.41	40.67±0.29	93.67±1.23	18.67±0.19	18.00±0.39	20.53±0.33	18.89±0.38	22.40±0.16	1.15±0.01
ECG-40	44.63±0.24	54.33±0.53	32.33±1.28	117.67±0.23	20.33±0.29	21.33±0.14	19.06±0.37	21.24±0.44	27.20±0.32	1.29±0.01

Note: average ± standard error

Continued Table 2 - Agronomic characteristics of lowland rice plants after exposure to 2,4-D dimethylamine herbicide

Lowland Rice	Flowering age (days)	Number of productive tillers	Panicle length (cm)	Fresh weight (g)	Dry weight (g)	Number of grains	Grain weight (g)	1,000-seed weight (g)	Yield (tons ha ⁻¹)	Yield decline (%)
ECG-0	46.33±0.14	18.33±0.23	24.93±0.06	182.32±3.63	57.21±3.12	954.33±10.25	18.13±0.19	26.87±0.64	4.53±0.05	28.05
ECG-1	49.33±0.60	12.33±0.21	23.17±0.04	120.92±0.52	29.26±0.16	766.67±21.34	14.57±0.41	19.68±0.47	3.64±0.10	42.20
ECG-2	42.00±1.11	11.00±0.18	24.23±0.19	93.82±1.06	32.03±1.11	925.00±40.70	23.91±1.75	27.21±0.65	5.98±0.44	5.13
ECG-3	44.00±0.16	10.00±0.55	22.90±0.15	64.99±3.95	17.70±1.08	412.00±21.27	7.83±0.40	16.57±0.40	1.96±0.10	68.94
ECG-4	46.33±0.14	13.67±0.26	22.73±0.12	97.44±1.22	24.20±0.20	719.00±13.10	13.66±0.25	22.40±0.53	3.42±0.06	45.79
ECG-5	47.67±0.34	9.33±0.32	24.23±0.19	72.58±4.06	26.15±2.27	467.00±32.34	8.87±0.61	17.61±0.42	2.22±0.15	64.79
ECG-6	41.67±0.21	13.67±0.50	23.37±0.20	98.76±2.48	24.40±0.80	679.00±14.02	12.90±0.27	21.64±0.52	3.23±0.07	48.81
ECG-7	44.00±0.09	11.67±0.26	23.03±0.04	78.73±0.68	21.17±0.10	549.33±2.22	10.44±0.04	19.18±0.46	2.61±0.01	58.58
ECG-8	46.00±0.18	15.00±0.18	23.37±0.12	125.88±2.05	33.82±0.95	935.67±42.16	17.78±0.80	26.52±0.63	4.44±0.20	29.45
ECG-9	35.33±0.99	13.33±0.14	20.60±0.08	142.95±1.07	45.14±1.77	398.67±25.82	7.57±0.49	16.31±0.39	1.89±0.12	69.94
ECG-10	44.00±0.24	14.33±0.14	24.63±0.14	114.07±1.56	28.12±0.46	801.00±27.96	15.22±0.53	23.96±0.57	3.80±0.13	39.61
ECG-11	44.33±0.36	12.00±0.16	23.77±0.03	97.23±2.19	23.47±0.62	691.00±28.56	13.13±0.54	21.87±0.52	3.28±0.14	47.90
ECG-12	46.00±0.31	9.00±0.24	23.00±0.09	71.66±2.32	17.80±0.63	572.00±11.47	10.87±0.22	19.61±0.47	2.72±0.05	56.87
ECG-13	48.00±0.39	10.67±0.23	26.03±0.10	116.37±0.69	25.77±0.20	564.67±12.71	10.73±0.24	19.47±0.46	2.68±0.06	57.43
ECG-14	46.00±0.24	8.00±0.31	23.23±0.05	55.41±2.26	14.32±0.52	217.67±15.06	4.14±0.29	12.88±0.31	1.03±0.07	83.59
ECG-15	45.67±0.28	13.67±0.23	23.07±0.16	115.38±1.61	36.26±1.15	642.33±12.94	12.20±0.25	20.94±0.50	3.05±0.06	51.57
ECG-16	29.33±0.05	10.00±0.16	20.97±0.08	118.30±1.52	35.56±0.33	453.67±21.77	8.62±0.41	17.36±0.41	2.15±0.10	65.79
ECG-17	45.67±0.28	10.67±0.19	24.27±0.07	90.45±0.48	29.42±0.84	679.00±6.07	12.90±0.12	21.64±0.52	3.23±0.03	48.81
ECG-18	50.67±0.05	8.33±0.28	24.13±0.14	95.37±1.46	25.51±0.36	493.33±32.77	9.37±0.62	18.11±0.43	2.34±0.16	62.80
ECG-19	48.00±0.24	11.33±0.05	23.93±0.13	110.95±2.45	35.89±1.27	721.67±10.95	13.71±0.21	22.45±0.54	3.43±0.05	45.59
ECG-20	43.67±0.14	13.00±0.48	24.93±0.19	107.00±2.93	22.73±0.49	603.67±22.16	11.47±0.42	20.21±0.48	2.87±0.11	54.49
ECG-21	44.67±0.14	16.00±0.09	24.43±0.11	150.56±1.92	34.01±0.39	603.00±25.61	11.46±0.49	20.20±0.48	2.86±0.12	54.54
ECG-22	43.00±0.48	18.33±0.34	25.43±0.08	168.87±5.02	57.72±3.11	579.67±27.63	11.01±0.52	19.75±0.47	2.75±0.13	56.29
ECG-23	48.67±0.29	9.00±0.18	22.23±0.16	75.79±1.04	25.36±1.08	323.67±7.67	6.15±0.15	14.89±0.36	1.54±0.04	75.60
ECG-24	27.67±0.21	18.33±0.05	19.73±0.09	139.34±0.69	61.73±0.27	264.33±12.98	5.02±0.25	13.76±0.33	1.26±0.06	80.07
ECG-25	27.00±0.33	15.00±0.24	20.73±0.04	150.45±3.01	54.31±0.76	536.67±7.92	10.20±0.15	18.94±0.45	2.55±0.04	59.54
ECG-26	45.00±0.31	11.00±0.00	22.40±0.17	69.33±0.67	19.07±0.23	414.67±10.63	7.88±0.20	16.62±0.40	1.97±0.05	68.74
ECG-27	30.67±0.19	14.33±0.10	21.17±0.08	152.53±0.14	55.36±0.97	477.67±16.52	9.08±0.31	17.82±0.43	2.27±0.08	63.99
ECG-28	46.33±0.21	10.00±0.18	22.40±0.09	68.16±3.33	18.47±0.69	466.33±20.84	8.86±0.40	17.60±0.42	2.22±0.10	64.84
ECG-29	46.00±0.41	9.33±0.44	20.80±0.09	64.79±2.03	17.84±0.59	488.33±32.45	9.28±0.62	18.02±0.43	2.32±0.15	63.18
ECG-30	49.00±0.62	8.67±0.66	23.60±0.09	134.31±4.19	30.71±1.01	678.33±45.55	12.89±0.87	21.63±0.52	3.22±0.22	48.86
ECG-31	43.67±0.43	17.00±0.48	24.37±0.21	138.93±5.26	41.47±1.30	499.67±15.37	9.49±0.29	18.23±0.43	2.37±0.07	62.33
ECG-32	48.00±0.65	11.33±0.84	25.73±0.09	113.98±9.03	19.21±1.37	653.33±74.45	12.41±1.41	21.15±0.50	3.10±0.35	50.74
ECG-33	49.00±0.18	15.33±0.28	24.13±0.19	159.88±3.58	65.34±2.79	817.33±15.19	15.53±0.29	24.27±0.58	3.88±0.07	38.38
ECG-34	45.67±0.05	18.00±0.41	23.10±0.05	150.16±2.93	69.42±3.17	984.67±10.03	19.34±0.27	28.08±0.67	4.84±0.07	23.25
ECG-35	47.33±0.29	10.00±0.31	23.10±0.12	81.19±2.69	28.95±1.75	486.00±19.18	9.23±0.36	17.97±0.43	2.31±0.09	63.36
ECG-36	45.67±0.34	12.33±0.14	24.87±0.16	95.48±1.25	24.23±0.50	674.67±9.30	12.82±0.18	21.56±0.51	3.20±0.04	49.13
ECG-37	43.00±0.27	17.33±0.10	24.43±0.16	161.73±1.08	38.61±0.53	799.33±18.30	15.19±0.35	23.93±0.57	3.80±0.09	39.73
ECG-38	48.67±0.19	13.67±0.14	26.37±0.04	105.32±0.55	25.47±0.44	654.67±26.01	12.44±0.49	21.18±0.51	3.11±0.12	50.64
ECG-39	43.00±0.31	14.00±0.24	23.37±0.13	69.16±1.44	22.07±0.68	437.67±20.02	8.32±0.38	17.06±0.41	2.08±0.10	67.00
ECG-40	44.67±0.05	15.00±0.27	23.77±0.07	157.96±2.20	54.03±2.35	572.33±20.89	10.87±0.40	19.61±0.47	2.72±0.10	56.85

Note: average ± standard error

Table 3 - Correlation values between agronomic characteristics with yield of lowland rice plants exposed to 2,4-D dimethylamine herbicide

No	Characteristics of lowland rice	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Plant height	1	0.374**	0.566**	0.639**	0.181*	0.282**	0.365**	0.462**	0.410**	-0.464**	-0.017	0.199*	0.021	0.215*
2	Number of leaves		1	0.621**	0.310**	-0.110	0.412**	0.636**	0.719**	0.581**	-0.356**	0.021	0.191*	0.007	0.164
3	Number of tillers			1	0.547**	0.017	0.259**	0.464**	0.588**	0.521**	-0.530**	-0.224*	0.060	-0.071	0.047
4	Leaf area				1	0.082	0.240**	0.290**	0.400**	0.351**	-0.667**	-0.204*	-0.023	-0.049	-0.020
5	Flag leaf length					1	0.147	0.003	0.130	0.100	0.173	0.281**	0.327**	0.140	0.390**
6	Flag leaf width						1	0.450**	0.519**	0.305**	-0.072	0.154	0.259**	0.012	0.206*
7	Number of productive tillers							1	0.750**	0.590**	-0.278**	0.046	0.434**	0.046	0.392**
8	Fresh weight								1	0.737**	-0.250**	0.084	0.485**	0.037	0.431**
9	Dry weight									1	-0.325**	-0.063	0.281**	-0.006	0.252**
10	Flowering age										1	0.461**	0.213*	0.181*	0.216*
11	Panicle length											1	0.291**	0.270**	0.301**
12	Number of grains												1	0.409**	0.963**
13	1,000-seeds weight													1	0.434**
14	Yield														1

Note: * and ** indicated the correlation is significant at the 0.05 and 0.01 levels. N: 123 samples

of tillers and 25.82% for the leaf area when compared to their respective measurements before spraying. However, the use of 2,4-D dimethylamine demonstrated safety for other growth and productivity aspects, particularly the yield rice. The yield of lowland rice in this study varied from 1.03 to 5.98 tons ha⁻¹. This observation was supported by the significant correlation found between various growth characteristics and the yield in lowland rice plants, except for the leaf area (negatively correlated). The rice yield was closely related to the photosynthate produced by the length and width of the flag leaf. This was further validated by the positive correlation and highly significant between the yield and the length and width of the flag leaf with values of 0.390** and 0.206*, respectively (Table 3). In addition, the length and width of the flag leaf were positively and significantly correlated to increase panicle length and the number of grains. These findings are supported by Rahman et al. (2013), a positive correlation between the flag leaf length in lowland rice plants and the panicle length, indicating a potential impact on grain yield. Additionally, Tian et al. (2015) indicated the significance of the flag leaf as the primary organ for photosynthesis, serving as the main source of assimilates required for plant growth and panicle development in lowland rice. Wang et al. (2020) further affirmed that the size of the flag leaf was closely related to the 1000-grain weight, grain weight per panicle, and other characteristics associated with grain yield. Regarding the response to the 2,4-D dimethylamine herbicide, Alridiwersah et al. (2022) reported that the total chlorophyll content of lowland rice plants exhibited inhibition from 2 to 7 days after spraying (DAS), followed by an increase at 14 DAS.

Alridiwersah et al. (2023b) also observed an increase in SPAD total chlorophyll in lowland rice plants exposed to 2,4-D dimethylamine herbicide at 6 DAS, indicating a form of recovery. Therefore, the effect of these herbicides on lowland rice plants appeared to be temporary, lasting for only a few days after spraying, after which recovery occurred, leading to increased biomass and grain yield.

The larger sizes of the upper epidermis, mesophyll, lower epidermis, and stomata density in barnyardgrass biotypes (ECG-1 to 40) compared to the ECG-0 when exposed to the 2,4-D dimethylamine (Table 4). The increased thickness of these histological leaf tissues affected the reduction in herbicide absorption and translocation in meristematic tissues. Consequently, when the herbicide was not adequately absorbed and translocated to the target site, it increased barnyardgrass biomass, as evidenced by higher dry weight, weed height, number of tillers, and other growth characteristics. This was confirmed by Table 5, that mesophyll tissue, lower epidermis, and stomata density were positively correlated with the fresh weight of barnyardgrass. These results were consistent with Huangfu et al. (2009), where weed populations with frequent exposure to herbicides (indicated resistance) generally exhibited thicker leaves and cuticles, along with a higher density of trichomes in the upper epidermal tissue compared to the control. Furthermore, the thicker upper epidermis in herbicide-resistant biotypes could reduce herbicide absorption and potentially alter the herbicide target site structure. Tampubolon et al. (2019) also reported on the significance of histological leaf measurements (upper epidermis, mesophyll, and lower epidermis) in weed resistance testing to herbicides.

Table 4 - Agro-histological characteristics of barnyardgrass after exposure to 2,4-D dimethylamine herbicide

Barnyardgrass	Number of tillers	Leaves length (cm)	Leaves width (cm)	Weed height (cm)	Flag leaf (cm)		Flowering age (days)	Seed maturity age (days)
					Length	Width		
ECG-0	4.33±0.68 a	13.18±2.06 ns	0.37±0.06 a	20.78±3.25 a	6.57±0.60 a	0.55±0.05 a	54.00±0.16 j	63.00±0.00 j-p
ECG-1	18.33±0.23 b-f	26.93±0.57 ns	0.75±0.01 b-g	51.48±1.02 b-d	14.73±0.12 c-h	1.14±0.01 b-h	41.33±0.21 b-j	52.33±0.59 b-l
ECG-2	24.33±0.66 efg	28.25±0.34 ns	0.71±0.01 b-g	52.20±0.44 b-d	14.60±0.27 c-h	0.99±0.01 b-h	41.00±0.36 b-j	46.67±0.10 a-e
ECG-3	18.33±0.14 b-f	31.33±0.23 ns	0.82±0.00 c-g	54.25±0.25 b-d	15.53±0.29 d-h	1.09±0.00 b-h	31.33±0.99 ab	46.67±0.10 a-e
ECG-4	20.00±0.90 b-f	33.60±0.64 ns	0.80±0.01 c-g	60.12±0.28 cde	14.90±0.12 c-h	1.16±0.00 b-h	40.67±0.69 b-j	51.00±0.63 b-i
ECG-5	34.00±0.41 gh	31.57±0.26 ns	0.71±0.00 b-g	52.70±0.41 b-d	13.27±0.16 b-g	0.97±0.00 b-h	37.67±0.14 a-g	48.00±0.31 a-f
ECG-6	17.33±0.36 b-f	36.07±0.50 ns	0.79±0.01 c-g	60.82±0.40 cde	17.87±0.18 gh	1.31±0.00 fgh	47.33±0.36 d-j	60.67±0.51 g-p
ECG-7	38.00±0.80 h	30.80±0.30 ns	0.80±0.01 c-g	57.45±0.08 cde	11.67±0.21 a-f	0.93±0.00 b-f	45.00±0.09 c-j	53.33±0.21 c-n
ECG-8	17.67±0.34 b-f	35.00±0.50 ns	0.84±0.01 c-g	58.98±0.45 cde	10.20±0.18 a-d	1.22±0.05 c-h	50.67±0.36 f-j	59.33±0.32 f-p
ECG-9	10.00±0.36 abc	30.13±0.59 ns	0.46±0.01 ab	48.20±0.86 b-d	13.07±0.57 b-g	1.32±0.06 gh	54.33±1.28 j	69.67±0.58 p
ECG-10	23.33±0.05 d-g	36.37±0.61 ns	0.74±0.01 b-g	63.88±0.26 e	11.73±0.20 a-f	0.90±0.00 b-e	35.67±0.21 a-e	46.00±0.00 a-d
ECG-11	21.67±0.46 c-f	30.92±0.54 ns	0.70±0.01 b-f	55.02±0.68 b-d	10.90±0.21 a-d	0.94±0.01 b-f	44.33±0.23 b-j	54.33±0.36 c-n
ECG-12	19.33±0.46 b-f	30.67±0.50 ns	0.76±0.00 c-g	64.00±0.08 e	11.50±0.40 a-f	1.00±0.02 b-h	27.00±2.03 a	38.33±1.93 a
ECG-13	16.67±0.45 b-f	36.65±0.46 ns	0.90±0.01 d-g	56.77±0.59 cde	14.13±0.29 b-g	1.19±0.01 b-h	46.67±0.14 d-j	54.67±0.21 c-n
ECG-14	28.33±0.21 fgh	35.70±0.22 ns	0.80±0.01 c-g	61.83±0.64 cde	12.50±0.13 b-f	0.87±0.01 a-d	42.33±0.38 b-j	51.33±0.45 b-j
ECG-15	14.33±0.73 a-e	30.17±0.53 ns	0.72±0.01 b-g	56.12±1.03 cde	16.37±0.14 e-h	1.24±0.01 d-h	48.33±0.05 e-j	58.00±0.16 e-o
ECG-16	11.33±0.44 a-d	29.78±0.17 ns	0.67±0.00 b-e	46.18±0.55 bcd	10.63±0.11 a-d	1.01±0.01 b-h	49.33±0.10 e-j	64.33±0.19 m-p
ECG-17	14.33±0.45 a-e	35.05±0.37 ns	0.81±0.00 c-g	63.57±0.19 de	19.53±0.18 h	1.35±0.00 h	51.33±0.41 g-j	62.33±0.28 i-p
ECG-18	16.33±0.32 a-f	30.57±0.18 ns	0.70±0.00 b-f	60.82±0.22 cde	13.90±0.15 b-g	1.11±0.01 b-h	45.33±0.14 c-j	57.00±0.16 d-o
ECG-19	23.33±0.46 d-g	28.73±0.12 ns	0.76±0.01 c-g	52.43±0.53 b-d	12.67±0.14 b-g	0.93±0.01 b-f	37.33±0.05 a-f	46.00±0.00 a-d
ECG-20	17.33±0.58 b-f	28.40±0.48 ns	0.68±0.02 b-f	49.53±1.27 b-d	10.90±0.09 a-d	0.98±0.01 b-h	42.33±0.36 b-j	49.33±0.52 a-f
ECG-21	15.00±0.63 a-e	30.33±0.28 ns	0.77±0.02 c-g	53.80±0.73 b-d	10.60±0.05 a-d	1.01±0.00 b-h	45.33±0.10 c-j	55.67±0.32 c-n
ECG-22	8.33±0.28 ab	29.48±0.50 ns	0.90±0.01 d-g	53.52±0.72 b-d	11.20±0.12 a-e	1.02±0.02 b-h	46.33±0.45 c-j	52.00±0.36 b-k
ECG-23	27.67±0.51 fgh	33.25±0.11 ns	0.79±0.01 c-g	54.32±0.02 b-d	12.63±0.15 b-g	0.99±0.00 b-h	38.00±0.09 a-h	46.00±0.00 a-d
ECG-24	19.33±0.68 b-f	31.88±0.82 ns	0.79±0.01 c-g	44.98±1.02 bc	13.80±0.23 b-g	1.10±0.01 b-h	48.00±0.09 d-j	65.00±0.09 nop
ECG-25	13.00±0.45 a-e	27.00±0.47 ns	0.61±0.00 a-d	38.12±0.37 b	13.03±0.15 b-g	1.00±0.01 b-h	45.67±0.23 c-j	64.00±0.00 l-p
ECG-26	23.00±0.41 d-g	32.15±0.05 ns	0.82±0.01 c-g	60.03±0.23 cde	14.53±0.29 c-h	1.23±0.02 d-h	53.00±0.63 ij	63.67±0.38 k-p
ECG-27	10.00±0.39 abc	33.35±0.79 ns	0.57±0.02 abc	44.45±1.05 bc	25.90±0.44 i	1.25±0.02 d-h	51.00±0.78 f-j	68.00±0.33 op
ECG-28	21.67±0.99 c-f	33.87±0.16 ns	0.77±0.00 c-g	60.05±0.24 cde	16.30±0.20 e-h	1.26±0.01 e-h	51.67±0.14 hij	63.00±0.00 j-p
ECG-29	24.33±0.45 efg	32.50±0.52 ns	0.89±0.01 d-g	61.85±0.52 cde	16.80±0.17 fgh	1.33±0.01 gh	49.00±0.24 e-j	61.67±0.21 h-p
ECG-30	16.00±0.18 a-f	24.82±1.13 ns	0.69±0.01 b-f	46.05±1.14 bcd	11.03±0.14 a-e	1.01±0.01 b-h	48.00±1.09 d-j	53.67±0.61 c-n
ECG-31	17.33±1.18 b-f	27.93±0.22 ns	0.94±0.01 efg	50.87±0.27 b-d	10.80±0.21 a-d	1.00±0.01 b-h	48.00±0.31 d-j	60.33±0.21 g-p
ECG-32	22.00±1.25 c-f	28.37±0.52 ns	0.97±0.02 fg	50.63±0.20 b-d	9.00±0.04 ab	0.83±0.01 ab	51.67±0.36 hij	61.67±0.21 h-p
ECG-33	12.00±0.54 a-e	34.18±0.09 ns	0.73±0.01 b-g	55.30±0.31 b-d	10.17±0.08 a-d	0.89±0.01 b-e	33.00±0.16 abc	41.00±0.00 ab
ECG-34	13.33±0.50 a-e	23.87±0.43 ns	0.70±0.02 b-f	44.20±0.81 bc	10.33±0.26 a-d	0.95±0.01 b-g	49.67±0.38 f-j	59.33±0.32 f-p
ECG-35	21.33±0.90 c-f	27.35±0.17 ns	0.77±0.01 c-g	56.93±0.29 cde	11.97±0.10 b-f	1.01±0.02 b-h	39.33±1.38 a-i	50.33±1.64 b-h
ECG-36	24.33±0.83 efg	32.22±0.54 ns	0.97±0.00 fg	49.23±0.37 b-d	11.03±0.27 a-e	0.91±0.02 b-e	49.33±0.60 e-j	60.67±0.51 g-p
ECG-37	11.67±0.14 a-d	37.43±0.70 ns	0.70±0.02 b-f	49.57±1.03 b-d	14.90±0.07 c-h	1.21±0.00 c-h	51.33±0.64 g-j	59.33±0.32 f-p
ECG-38	27.67±0.29 fgh	31.98±0.08 ns	1.01±0.01 g	52.32±0.31 b-d	10.07±0.09 abc	1.01±0.01 b-h	48.00±0.24 d-j	59.33±0.32 f-p
ECG-39	28.33±0.34 fgh	35.50±0.68 ns	0.85±0.01 c-g	61.12±0.74 cde	12.60±0.25 b-g	0.84±0.00 abc	34.33±0.73 a-d	44.33±0.26 abc
ECG-40	11.33±0.63 a-d	26.95±0.40 ns	0.73±0.02 b-g	45.20±0.83 bc	12.77±0.26 b-g	1.10±0.01 b-h	44.33±0.92 b-j	53.00±0.94 c-m
CV (%)	32.44	20.18	19.46	16.25	20.15	17.78	15.14	10.51

Note: average followed by different letters indicates a significant effect in DMRT at 5% level ± standard error. ECG-0= comparison population; ECG-1 to ECG-40= barnyardgrass biotypes. Ns: not significant. CV: coefficient of variation

Continued Table 4 - Agro-histological characteristics of barnyardgrass after exposure to 2,4-D dimethylamine herbicide

Barnyardgrass	Panicle length (cm)	Fresh weight (g)	Dry weight (g)	Leaf histological size (μm)			Stomatal density (n/μm)
				Upper epidermis	Mesophyll	Lower epidermis	
ECG-0	9.57±0.76 a	53.82±4.24 a	7.70±1.20 a	10.91±1.70 a	32.30±2.58 a	10.46±1.09 a	12.62±1.15 a
ECG-1	14.40±0.08 fg	181.51±2.58 d-i	55.08±0.98 a-g	34.55±0.47 m	87.55±0.91 l-o	16.35±0.30 c-g	27.34±0.21 f
ECG-2	12.13±0.11 a-g	250.91±3.49 i-m	114.20±7.27 fgh	23.64±0.32 e-k	69.87±0.73 ghi	18.59±0.34 e-j	38.33±0.30 klm
ECG-3	13.00±0.05 a-g	259.21±5.67 j-n	64.90±1.33 a-h	18.47±0.25 b-h	41.52±0.43 b	21.43±0.39 h-m	33.07±0.25 hi
ECG-4	13.93±0.09 b-g	251.09±4.53 i-m	83.73±3.44 b-h	17.92±0.24 b-h	49.67±0.52 bcd	16.99±0.31 c-g	52.85±0.41 t
ECG-5	14.23±0.07 efg	273.28±2.79 l-o	104.53±7.88 e-h	19.17±0.26 b-i	151.32±1.57 v	14.76±0.27 b-e	41.66±0.32 no
ECG-6	13.13±0.09 a-g	263.58±3.80 k-n	77.86±2.55 b-h	18.66±0.25 b-i	96.14±1.00 op	25.57±0.47 mno	35.31±0.27 jk
ECG-7	14.03±0.12 c-g	325.73±1.57 no	79.39±0.60 b-h	13.91±0.19 ab	107.21±1.11 qrs	21.77±0.40 j-n	43.61±0.34 op
ECG-8	14.53±0.14 fg	191.21±4.57 f-j	43.44±0.31 a-e	21.12±0.29 c-j	52.14±0.54 cd	29.58±0.54 p	42.33±0.33 no
ECG-9	11.67±0.24 a-f	117.78±5.40 a-e	37.80±2.28 a-e	34.97±0.48 m	116.96±1.22 st	17.85±0.33 d-j	30.92±0.24 gh
ECG-10	13.87±0.26 b-g	234.61±2.94 h-m	48.62±0.22 a-f	15.82±0.22 a-d	105.32±1.10 pqr	18.56±0.34 e-j	49.63±0.38 qr
ECG-11	13.10±0.06 a-g	197.18±2.22 g-k	40.18±0.35 a-e	17.62±0.24 a-g	89.45±0.93 mno	14.14±0.26 a-e	23.81±0.18 cde
ECG-12	10.23±0.22 abc	272.61±2.45 l-o	117.37±9.59 gh	15.23±0.21 abc	75.13±0.78 h-k	17.32±0.32 c-i	26.75±0.21 ef
ECG-13	14.20±0.07 d-g	165.48±1.66 b-h	49.19±2.92 a-f	22.43±0.31 c-k	80.58±0.84 j-m	21.56±0.39 i-n	59.46±0.46 u
ECG-14	14.63±0.09 fg	286.32±2.51 l-o	62.35±0.85 a-g	20.89±0.29 b-j	88.75±0.92 mno	19.47±0.36 f-k	41.30±0.32 mno
ECG-15	13.23±0.18 a-g	237.59±1.56 h-m	70.89±3.01 a-h	17.92±0.24 b-h	108.04±1.12 qrs	26.83±0.49 op	27.78±0.21 f
ECG-16	13.10±0.07 a-g	111.34±2.04 a-d	21.68±0.44 ab	34.16±0.47 m	89.33±0.93 mno	21.89±0.40 j-n	33.63±0.26 hij
ECG-17	14.53±0.04 fg	326.10±2.19 no	81.59±2.14 b-h	18.97±0.26 b-i	71.76±0.75 g-j	25.72±0.47 no	51.58±0.40 rt
ECG-18	11.47±0.16 a-f	245.36±1.93 i-m	93.83±6.10 d-h	25.04±0.34 h-l	114.17±1.19 rst	15.39±0.28 b-f	26.64±0.21 def
ECG-19	13.60±0.10 b-g	230.66±2.20 h-l	71.69±3.47 a-h	23.92±0.33 f-l	77.64±0.81 i-l	14.78±0.27 b-e	36.37±0.28 jkl
ECG-20	14.00±0.09 c-g	183.36±4.99 d-i	59.67±4.27 a-g	21.27±0.29 c-j	84.15±0.88 k-n	16.96±0.31 c-g	23.64±0.18 cd
ECG-21	14.50±0.15 fg	169.00±0.51 c-h	38.64±1.40 a-e	24.56±0.34 g-l	65.81±0.68 fgh	20.04±0.37 g-l	36.08±0.28 jk
ECG-22	14.67±0.10 fg	154.54±1.23 b-g	31.59±0.13 a-d	21.25±0.29 c-j	69.79±0.73 ghi	23.92±0.44 l-o	44.29±0.34 op
ECG-23	14.67±0.11 fg	236.10±3.17 h-m	47.41±0.91 a-f	18.46±0.25 b-h	101.16±1.05 pq	17.09±0.31 c-g	34.11±0.26 hij
ECG-24	12.07±0.07 a-g	93.11±1.34 ab	23.77±0.65 ab	26.52±0.36 jkl	43.32±0.45 bc	22.87±0.42 k-o	48.67±0.37 q
ECG-25	12.77±0.08 a-g	96.41±1.36 abc	24.77±0.16 abc	29.15±0.40 klm	79.71±0.83 i-m	17.31±0.32 c-i	23.83±0.18 cde
ECG-26	13.97±0.23 b-g	340.09±4.15 o	129.18±6.83 h	19.01±0.26 b-i	104.48±1.09 pqr	11.35±0.21 ab	30.90±0.24 gh
ECG-27	13.50±0.12 b-g	101.01±3.21 abc	31.33±1.57 a-d	16.93±0.23 a-f	132.52±1.38 u	15.24±0.28 b-f	45.63±0.35 p
ECG-28	14.80±0.14 fg	305.60±4.31 mno	92.74±2.20 c-h	42.15±0.58 n	83.44±0.87 k-n	16.19±0.30 c-g	37.29±0.29 kl
ECG-29	14.63±0.02 fg	337.25±4.05 o	80.84±1.43 b-h	26.52±0.36 jkl	73.35±0.76 hij	23.39±0.43 k-o	42.34±0.33 no
ECG-30	13.17±0.19 a-g	124.26±2.77 a-f	28.04±0.89 a-d	30.87±0.42 lm	52.02±0.54 cd	13.11±0.24 abc	39.30±0.30 lmn
ECG-31	10.40±0.06 a-e	127.30±2.43 b-g	41.29±3.22 a-e	22.61±0.31 d-k	62.86±0.65 efg	16.66±0.30 c-g	31.59±0.24 gh
ECG-32	10.90±0.09 a-f	188.38±5.86 e-j	35.41±1.26 a-d	24.26±0.33 g-l	91.62±0.95 no	15.04±0.28 b-e	33.64±0.26 hij
ECG-33	12.67±0.12 a-g	105.28±7.18 abc	31.61±0.18 a-d	21.30±0.29 c-j	88.85±0.92 mno	18.12±0.33 d-j	29.47±0.23 fg
ECG-34	11.00±0.15 a-f	119.54±0.79 a-f	47.06±3.67 a-f	17.66±0.24 a-g	80.36±0.84 j-m	17.18±0.31 c-h	23.32±0.18 c
ECG-35	10.03±0.30 ab	295.69±1.02 l-o	113.32±7.07 fgh	27.46±0.37 jkl	113.60±1.18 rst	14.46±0.26 a-e	19.79±0.15 b
ECG-36	10.43±0.04 a-e	180.80±2.86 d-i	54.67±2.95 a-g	26.66±0.36 jkl	54.28±0.56 de	13.05±0.24 abc	33.37±0.26 hij
ECG-37	15.97±0.15 g	98.08±3.80 abc	32.61±2.20 a-d	16.59±0.23 a-e	112.57±1.17 rst	19.86±0.36 g-l	28.64±0.22 fg
ECG-38	10.27±0.11 a-d	179.39±1.20 d-i	53.06±2.67 a-g	23.29±0.32 e-k	59.19±0.62 def	13.01±0.24 abc	39.41±0.30 lmn
ECG-39	11.73±0.25 a-f	296.85±1.15 l-o	81.98±1.89 b-h	25.71±0.35 i-l	72.35±0.75 g-j	15.67±0.29 b-g	41.33±0.32 mno
ECG-40	13.73±0.20 b-g	138.90±5.50 b-g	32.98±0.10 a-d	21.22±0.29 c-j	118.51±1.23 t	13.82±0.25 a-d	51.98±0.40 rt
CV(%)	15.04	18.26	21.65	15.89	6.40	12.25	4.74

Note: average followed by different letters indicates a significant effect in DMRT at 5% level ± standard error. ECG-0: comparison population; ECG-1 to ECG-40: barnyardgrass biotypes; ns: not significant; CV: coefficient of variation

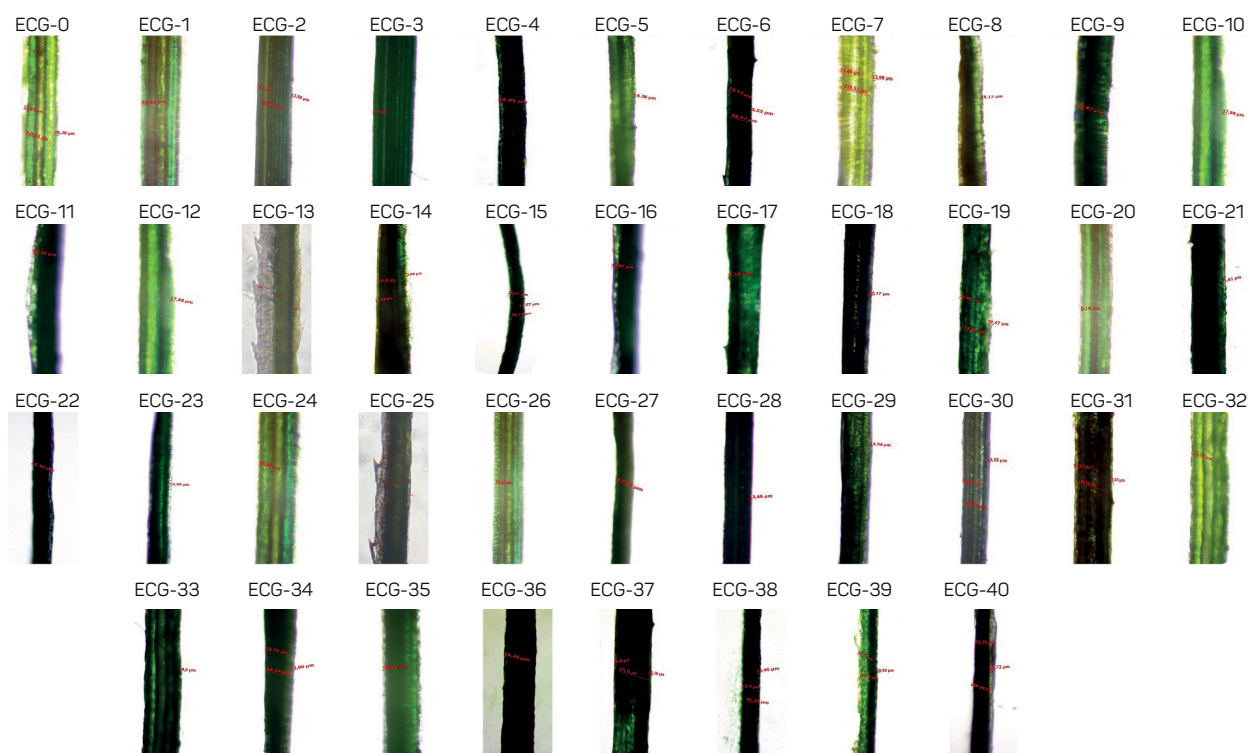


Figure 3 - Leaf histological tissue cross-section of barnyardgrass after exposure to herbicide 2,4-D dimethylamine at 1 WAS. ECG-0= comparison population; ECG-1 to ECG-40= barnyardgrass biotypes

The application of the 2,4-D dimethylamine at a dose of 1,080 g ai ha⁻¹ only accelerated the flowering and seed maturity ages of the ECG-12 biotype compared to the other barnyardgrass biotypes and the comparison population (Table 4). The growth characteristics of barnyardgrass from Serdang Bedagai after exposure to the 2,4-D dimethylamine herbicide, exhibited significant variation and were more favorable than those of the comparison population. This suggested that the weed has developed a non-target site resistance mechanism to the 2,4-D dimethylamine, characterized by larger sizes of upper epidermis, mesophyll, and lower epidermis tissues. Consequently, the herbicide was unable to be adequately absorbed and translocated to the target site through the phloem pathway (Grossmann, 2010; Schulz, Segobye, 2016). This lack of proper translocation affected the number of tillers, leaf length and width, plant height, length and width of the flag leaf, panicle length, fresh and dry weight, and number of seeds, all of which were higher than those observed in the comparison population. These growth characteristics have a direct influence on the age of seed maturity and the seeds produced. The seeds produced and the age of seed maturity in barnyardgrass biotypes ranged from 1,298.00 to 9,457.33 seeds and 38.33 to 69.67 days, respectively, potentially contributing to the widespread seedbank in lowland rice fields. Although the seeds produced by barnyardgrass biotypes was lower than that reported by Tahir (2016), ranging from 7,186 to 71,494 seeds, the age

of seed maturity was notably shorter, ranging from 80 to 94 days. This shorter age of seed maturity combined with the abundance of seeds produced by the barnyardgrass biotypes has failed farmers to control them. These resistant biotypes require breaking the cycle to prevent yield losses of lowland rice plants in Serdang Bedagai District.

5. Conclusions

Higher doses of 2,4-D dimethylamine herbicide was considered safe for lowland rice growth and has led to yields ranging from 1.03 to 5.98 tons ha⁻¹, it became ineffective in suppressing the growth and seed production of barnyardgrass biotypes. This study represented the first information linking the effect of higher doses of the 2,4-D dimethylamine herbicide causing enlarged tissue size of the upper epidermis, mesophyll, and lower epidermis compared to the comparison population (ECG-0). The seeds produced by the barnyardgrass biotypes ranged from 1.13 to 8.20 times compared to the comparison population. These results emphasized the urgent need for alternative management strategies to prevent the spread of the barnyardgrass biotypes in lowland rice fields.

Author's contributions

KT, and A: conceptualization. KT, and MB: design and methodology. KT, and NEM: data collection and analyses.

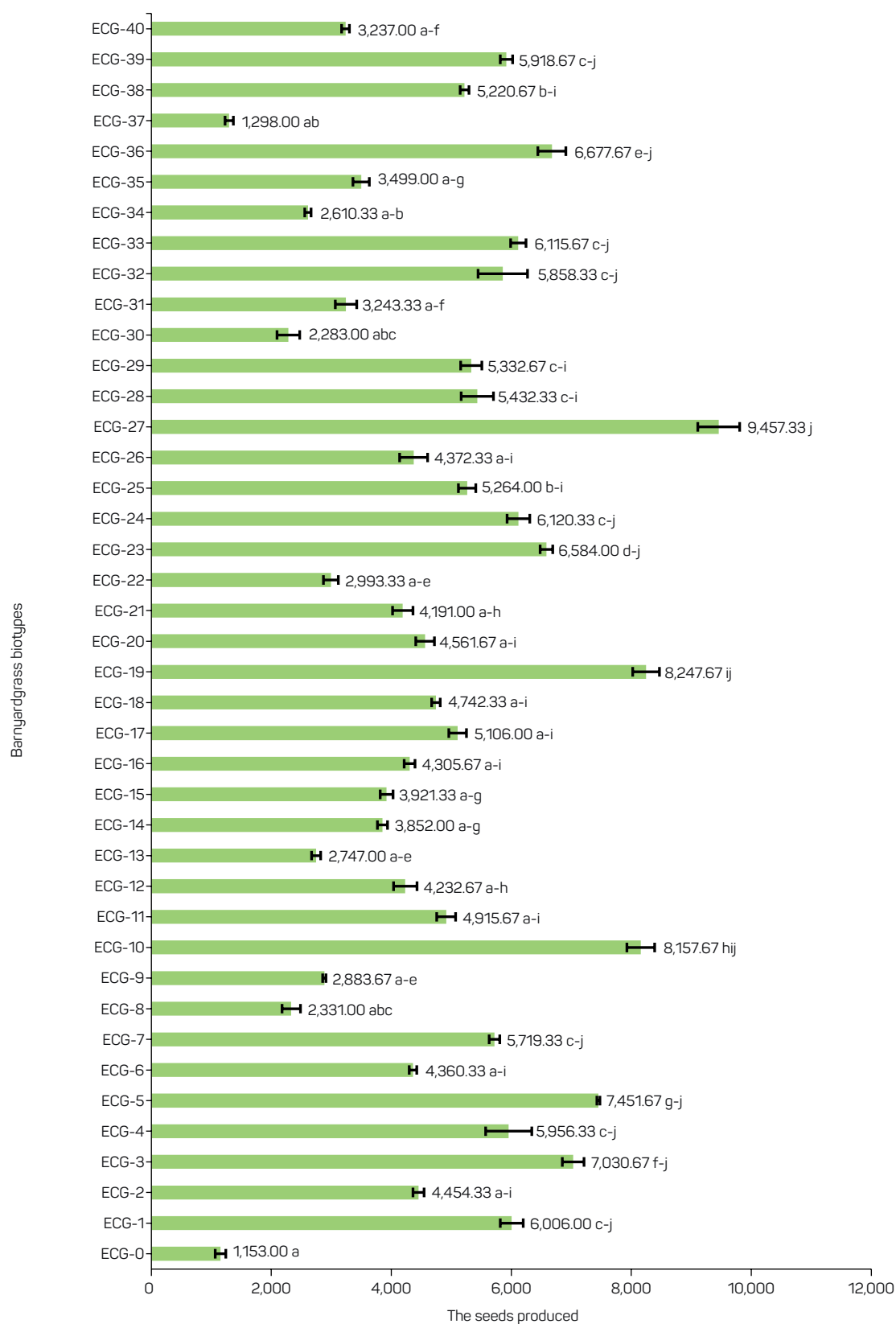


Figure 4 - The seed produced of barnyardgrass biotypes after the 2,4-D dimethylamine sprayed at a dose of 1,080 g ai ha⁻¹ in lowland rice fields

Table 5 - Correlation values between agro-histological characteristics with seed produced of of barnyardgrass after exposed to 2,4-D dimethylamine

No	Barnyardgrass Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Weed height	1	0.387*	0.577**	0.335*	-0.262	-0.025	0.260	0.194	0.106	0.154	0.175	0.794**	0.630**	0.047
2	Number of tillers		1	0.153	0.300	-0.146	0.098	-0.238	0.145	-0.138	-0.377*	-0.002	0.661**	0.521**	0.413**
3	Leaves length			1	0.261	-0.303	-0.011	0.375*	0.384*	0.326*	0.259	0.414**	0.260	0.093	0.095
4	Leaves width				1	-0.195	-0.535**	0.111	0.179	-0.259	-0.205	-0.105	0.238	0.105	-0.101
5	Upper epidermis					1	-0.082	-0.201	-0.097	-0.086	0.133	-0.127	-0.145	-0.125	-0.028
6	Mesophyll						1	-0.248	-0.076	0.250	0.104	0.117	0.104	0.161	0.193
7	Lower epidermis							1	0.222	0.192	0.405**	0.385*	0.049	-0.136	-0.276
8	Stomatal density								1	0.271	0.204	0.339*	0.120	-0.095	0.227
9	Flag leaf length									1	0.686**	0.323*	0.201	0.205	0.310*
10	Flag leaf width										1	0.347*	0.117	0.133	-0.185
11	Panicle length											1	0.124	-0.102	-0.039
12	Fresh weight												1	0.840**	0.230
13	Dry weight													1	0.105
14	Seed produced														1

Note: * and ** indicated the correlation is significant at the 0.05 and 0.01 levels. n: 123 samples

A, and NEM: seed collecting. KT, A, and MB: interpretation of data processing and literature search. All authors: writing, review and editing.

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