

Seed pre-soaking with brassinolide enhances photosynthetic resistance of foxtail millet seedlings under mesosulfuron-methyl + iodosulfuron-methyl-sodium stress

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ABSTRACT

The mixture of mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) as post-emergence dual-purpose herbicide exhibits phytotoxicity in foxtail millet (*Setaria italica* (L.) P. Beauv.). Reducing ME+IMS stress in foxtail millet is therefore of practical significance. This study assessed the effects of seed pre-soaking with different concentrations of brassinolide (0.01, 0.1, 1.0 and 2.0 mg L⁻¹) on foxtail millet grown under ME+IMS (2.81+0.56 g ai ha⁻¹) stress. The agronomic characteristics, photosynthetic pigment, gas exchange parameters, chlorophyll fluorescence and P₇₀₀ parameters of two foxtail millet cultivars ('Jingu 21' and 'Zhangza 5') were analyzed 7 d after ME+IMS treatment at the 5th leaf stage. Compared with ME+IMS treatment alone, brassinolide treatment within a concentration range of 0.1-1.0 mg L⁻¹ had a positive effect on all plant parameters to varying degrees. Pre-soaking of seeds with 0.1 mg L⁻¹ brassinolide increased the leaf area (41.8%), fresh mass (179.2%), net photosynthetic rate (P_n, 38.4%), transpiration rate (T_r, 61.5%), stomatal conductance (g_s, 103.1%), photosystem II maximum quantum yield (F_v/F_m, 8.6%), effective quantum yield (Y(II), 16.0%), electron transport rate (ETR(II), 17.8%), photosystem I photochemical quantum yield (Y(I), 16.7%) and electron transport rate (ETR(I), 38.7%) in 'Zhangza 5' leaves under ME+IMS stress. An increase in plant height (53.0%), total chlorophyll (38.3%), carotenoid contents (40.0%), and maximal P₇₀₀ change (P_m, 34.3%) were also observed following 1.0 mg L⁻¹ brassinolide treatment compared with ME+IMS in 'Jingu 21'. Comprehensive analysis showed that pre-sowing seed treatment with 0.1-1.0 mg L⁻¹ brassinolide could enhance the growth and photosynthetic resistance of foxtail millet seedlings to ME+IMS stress.

Key words: Brassinolide, photosynthetic physiology, seed presoaking, *Setaria italica*, sulfonylurea herbicide.

INTRODUCTION

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is an annual cereal and fodder crop of the Gramineae family originating from North China (Jones and Liu, 2009). Because it possesses many elite traits such as high resistance to abiotic stress, foxtail millet is widely grown in arid and semi-arid areas across 26 countries and regions, including China, India, USA, and Nigeria (Sharma and Niranjana, 2018). The total annual growing area of foxtail millet is approximately two million hectares, most of which is located in the North China Plain, Northeast China, and Northwest Loess Plateau. Foxtail millet grain is a staple food, while its straw is used as high-quality feed for animals such as sheep, goats, and cows (Austin, 2006; Li et al., 2021).

Weeds in foxtail millet fields seriously impact production. Despite herbicides are widely applied in modern agriculture of rice (Butt et al., 2020), maize and soybean (Nandula, 2019), few can safely be applied in foxtail millet. The commercial mixture of mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) is a highly selective post-emergence herbicide developed by Bayer Crop Science (Germany). It can control the majority of gramineous and some broadleaf weeds, working well in wheat fields when applied at a recommended dose (Guo et al., 2009). Although foxtail millet and wheat belong to the same Gramineae family, previous study revealed that ME+IMS applied at the recommended effective dose exhibited remarkable phytotoxicity in foxtail millet (Zhang et al., 2020a). Reducing ME+IMS stress in foxtail millet is therefore of practical significance.

Brassinolide is a plant steroid that is known to enhance plant resistance against abiotic stresses such as insecticides and herbicides (Sharma et al., 2015). Moreover, there is evidence that brassinolide application can modulate other endogenous phytohormones as well as gene expression (Yin et al., 2019) and activities of antioxidative enzymes (Niu et al., 2016) in plants. Improvements in the chlorophyll content, regulation of osmo-protectants, and enhanced net photosynthetic rates have been revealed (Lv et al., 2020). Brassinolide treatment also decreases levels of malondialdehyde, alleviating damage to the chloroplasts and mitochondria in plants under stress (Sun et al., 2020; Zhang et al., 2020b). Meanwhile, in foxtail millet, foliar spraying of brassinolide was found to ameliorate ME+IMS toxicity (Yuan et al., 2017).

Foxtail millet seeds are small in size with a low ability to break through the soil surface after germination. Pre-soaking seed treatment is an effective approach of facilitating germination. For tomato (Ahammed et al., 2012) and beans (Cheng et al., 2015), brassinolide treatment was found to improve germination capacity of seeds, and eliminate rot and necrosis of roots and sprouts, resulting in uniform and robust seedlings. Moreover, seed soaking with brassinolide could increase root activity in rice, while decreasing the absorption of herbicides and attenuating their inhibitory effect on photosynthesis in rice leaves (Mu et al., 2002; Zhou et al., 2003). However, the effects of seed pre-soaking treatment with brassinolide on foxtail millet seedlings under ME+IMS toxicity remain unclear.

The aim of the present study was to assess the physiological mechanisms underlying the effects of seed soaking with different concentrations of brassinolide before sowing in foxtail millet seedlings grown under ME+IMS stress, and screen suitable brassinolide concentration. The results provide a theoretical foundation and technical approach for safe application of the sulfonylurea herbicide ME+IMS in foxtail millet fields, aiding appropriate use of the plant growth regulator brassinolide to ameliorate herbicide phytotoxicity.

MATERIALS AND METHODS

Materials and experiment design

The study was conducted in the greenhouse of Shanxi Agricultural University in Taigu, Shanxi Province, China. Seeds of foxtail millet (*Setaria italica* (L.) P. Beauv.) cultivars, conventional 'Jingu 21' and hybrid 'Zhangza 5', were provided by the Institute of Economic Crops, Shanxi Academy of Agricultural Sciences (Fenyang, China), and Zhangjiakou Academy of Agricultural Sciences, Hebei Province (Zhangjiakou, China), respectively.

The two acetolactate synthase (ALS) inhibiting herbicides of mesosulfuron-methyl (methyl 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-4-(methanesulfonamidomethyl)benzoate) + iodosulfuron-methyl-sodium sodium ((5-iodo-2-methoxycarbonylphenyl)sulfonyl-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoyl]azanide) were studied in a commercial mixture. Commercial formulations of mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) were applied at $2.81 + 0.56 \text{ g ai ha}^{-1}$ (Atlantis WG, 3% w/v mesosulfuron-methyl + 0.6% w/v iodosulfuron-methyl-sodium, Bayer CropScience, St. Louis, Missouri, USA), a quarter of recommended field rates given by the manufacturer. The seeds of foxtail millet were surface disinfected with 0.1% KMnO_4 then rinsed three times in distilled water. The surface disinfected seeds were soaked for 12 h in different effective concentrations (0.01, 0.1, 1.0, and 2.0 mg L^{-1}) of brassinolide wettable powder (0.01%; Chengdu New Sun Crop Science, Chengdu, China).

The experiment followed a split-plot design with three replicates. Main plots contained the two cultivars, while sub-plots contained five chemical treatments with water as a control (CK); M: ME+IMS alone; BR0.01+M: 0.01 mg L^{-1} brassinolide and ME+IMS; BR0.1+M: 0.1 mg L^{-1} brassinolide and ME+IMS; BR1+M: 1.0 mg L^{-1} brassinolide and ME+IMS; BR2+M: 2.0 mg L^{-1} brassinolide and ME+IMS. Each replicate consisted of three plastic pots 13 cm in diameter and 15 cm in height.

Pots were filled with growth substrate consisting of a 1:2 mixture of sand and loam soil with moderate fertility. Forty seeds per cultivar pretreated with or without brassinolide were sown to a depth of 1 cm equidistantly in a pot. Pots were then placed in a greenhouse maintained at day/night temperatures of 25/17 °C. Seedlings were thinned to seven plants per pot at the three-leaf stage. A hand-held sprayer was used to apply ME+IMS at $2.81 + 0.56 \text{ g ai ha}^{-1}$ plus 0.4% (v/v) alkyl ethyl sulfonate as adjuvant at the five-leaf stage. An equal amount of water was sprayed in the control treatment. Seven days after ME+IMS treatment, seedlings showing uniform growth (three per treatment) were selected and the penultimate leaf was sampled to assay physiological and biochemical parameters.

Measurement of agronomic characteristics

Plant height was measured from the base of the plant to the flag leaf, while leaf length and width were recorded as the longest and widest distances of the penultimate leaf. Plant height, leaf length, and leaf width were all measured with a ruler. Leaf area was then calculated as follows: Leaf area = leaf length \times leaf width \times 0.75 (Yuan et al., 2017). The fresh mass of the whole plant was weighed using an EL104 ten-thousand analytical balance (Mettler-Toledo, LLC, Shanghai, China).

Measurement of photosynthetic pigment contents and gas exchange parameters

Photosynthetic pigments were extracted from penultimate leaf samples in 96% (v/v) alcohol according to Yuan et al. (2017) with some adjustments. Briefly, fresh penultimate leaf samples (0.1 g each) were soaked in 10 mL alcohol (96%, v/v) then stored in the dark for 24 h. The supernatants were then collected for measurement of pigment contents using the absorbance at 665 nm for chlorophyll a (Chl a), 649 nm for chlorophyll (Chl b), and 470 nm for carotenoids (Car) using a 756PC-UV-VIS spectrophotometer (Shanghai Spectrum Instruments, Shanghai, China). The Chl a, Chl b, and Car contents were then calculated using the following equations:

$$\begin{aligned} C_{\text{Chl a}} &= 13.95 \times \text{OD}_{665} - 6.88 \times \text{OD}_{649} \\ C_{\text{Chl b}} &= 24.96 \times \text{OD}_{649} - 7.32 \times \text{OD}_{665} \\ C_{\text{Car}} &= 1000 \times \text{OD}_{470} - 2.05 \times C_{\text{Chl a}} - 114.8 \times C_{\text{Chl b}} / 245 \\ \text{Pigment content (mg g}^{-1} \text{ F}_m) &= C \times V_T \times n / \text{FM} \times 1000 \end{aligned}$$

where C is the pigment concentration (mg L^{-1}), FM is the fresh mass (g), V_T is the total volume of the extract (mL), and n is the dilution factor.

Photosynthetic gas exchange parameters were measured in the penultimate leaf using a CI-340 portable photosynthesis system (CID Bio-Science, Camas, Washington, USA). Measurements of net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), and intercellular CO_2 concentrations (C_i) were conducted simultaneously between 09:00 and 11:00 h under the following conditions: light intensity $800 \pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$; CO_2 concentration $400 \pm 0.4 \mu\text{mol mol}^{-1}$; air flow rate $750 \mu\text{mol s}^{-1}$; and air temperature $25 \pm 0.4 \text{ }^\circ\text{C}$.

Measurement of chlorophyll fluorescence and P_{700} parameters

Chlorophyll fluorescence (Fluo) and P_{700} parameters were obtained according to Yuan et al. (2013) after seedlings were placed in the dark for 30 min. Simultaneous measurements were performed using a DUAL-PAM-100 system (Walz, Effeltrich, Germany) with the automated Induction and Recovery Curve routine provided by the DualPAM software. First, the fluorescence induced curve (Slow Kinetics) was obtained in the “Fluo + P_{700} mode” then the kinetics of chlorophyll fluorescence induction and P_{700} oxidation were recorded simultaneously.

The initial fluorescence (F_o) was recorded then the maximum fluorescence (F_m) was measured using the saturation pulse method. The maximum quantum yield of photosystem II (PSII) was then calculated as: $F_v/F_m = (F_m - F_o)/F_m$. Other energy dissipation parameters of PSII were estimated using the DualPAM software. Three complementary quantum yields of energy conversion in PSII were calculated (Kramer et al., 2004): Effective quantum yield defined, $Y(\text{II}) = (F'_m - F)/F'_m$; yield of non-photochemical losses via non-regulated pathways, $Y(\text{NO}) = 1/(NPQ + 1 + qL \times (F_m/F_o - 1))$, $NPQ = F_m/F'_m - 1$, $qL = qP \times F'_o/F$, $qP = (F'_m - F)/(F'_m - F'_o)$; and the quantum yield of regulated energy dissipation, $Y(\text{NPQ}) = 1 - Y(\text{II}) - Y(\text{NO})$. The apparent electron transfer rate of PSII in light was expressed as $\text{ETR}(\text{II}) = \text{PAR} \times 0.84 \times 0.5 \times Y(\text{II})$, and used to measure electron transfer of C fixation resulting from the photochemical reactions.

The action center chlorophyll of photosystem I (PSI), P_{700} , is a dimer of one Chl a and one Chl a'. The P_{700} absorbance parameters reflect the photochemistry of PSI. The maximum P_{700} change (P_m) was determined by application of saturation

pulses (SP) after far-red preillumination. Actinic illumination was then provided and SP were given every 20 s, with the same pulse serving for fluorescence and P_{700} analysis. The P_{700} oxidation was monitored based on absorbance changes in the near-infrared spectral region (830-875 nm) (Schreiber and Klughammer, 2008). The maximum P_{700} signal observed upon full oxidation was denoted as P_m . The photochemical quantum yield of PSI was estimated as $Y(I) = (P'_m - P)/P_m$, while the quantum yield of non-photochemical energy dissipation due to acceptor-side limitation was calculated as $Y(NA) = (P'_m - P_m)/P'_m$. The quantum yield of non-photochemical energy dissipation due to donor-side limitation in PSI was calculated as $Y(ND) = (P - P_o)/P_m$. The sum of the three quantum yields was then determined as $Y(I) + Y(ND) + Y(NA) = 1$. The ETR of PSI (ETR(I)) was provided by the DualPAM software.

Statistical analysis

Data are presented as means \pm standard deviations. The Data Processing System (DPS 7.05) (Tang and Zhang, 2013) was used to carry out statistical analysis of the data. Duncan's multiple range test was used to evaluate the significance of differences between treatments in each cultivar at a 5% probability level. All experiments were performed at least in triplicate.

RESULTS

Agronomic characteristics

Compared with the control, M treatment decreased plant height, leaf area, and fresh mass by 31.5%, 34.8%, and 67.8% in 'Zhangza 5', and 41.5%, 63.3% and 38.5% in 'Jingu 21', respectively (Table 1). With increasing brassinolide, plant height, leaf area, and fresh mass of both cultivars first increased then decreased. Compared with M treatment, BR0.1+M significantly increased the leaf area and fresh mass of 'Zhangza 5' by 41.8% and 179.2%, respectively, while plant height was highest under BR1+M. Meanwhile, compared with M treatment, BR1+M significantly improved the plant height and fresh mass of 'Jingu 21' by 53.0% and 77.3%, respectively, while BR0.1+M dramatically increased the leaf area by 113.7%.

Photosynthetic pigments

Compared with the control, M treatment decreased chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll contents by 30.9%, 45.8%, 30.8%, and 34.3%, in the leaves of 'Zhangza 5' and by 27.5%, 46.4%, 35.5%, and 31.9% in 'Jingu 21', respectively (Table 2). Brassinolide soaking treatment had a significant effect on the photosynthetic pigment contents in 'Zhangza 5', with an increase in chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll of 44.6%, 61.5%, 44.4%, and 46.4% under BR1+M compared with M treatment, respectively. In 'Jingu 21', respective increases of 36.4%, 46.7%, 40.0%, and 38.3% were observed under BR1+M compared to M treatment.

Table 1. Effect of seed presoaking with different concentrations of brassinolide on growth parameters in two foxtail millet cultivars under mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) treatment.

Cultivars	Treatment	Height cm	Leaf area cm ²	Fresh mass g
Zhangza 5	CK	15.73 \pm 2.50a	20.17 \pm 3.29a	1.49 \pm 0.12a
	M	10.77 \pm 0.38b	13.16 \pm 1.28b	0.48 \pm 0.05d
	BR0.01+M	11.60 \pm 0.40b	16.43 \pm 1.56ab	0.95 \pm 0.15c
	BR0.1+M	12.27 \pm 0.25b	18.66 \pm 0.31a	1.34 \pm 0.05ab
	BR1+M	12.33 \pm 0.29b	15.39 \pm 4.73ab	0.98 \pm 0.17c
	BR2+M	12.10 \pm 0.79b	16.47 \pm 1.16ab	1.25 \pm 0.13b
Jingu 21	CK	19.38 \pm 0.25a	25.00 \pm 1.83a	1.43 \pm 0.33a
	M	11.33 \pm 0.76d	9.18 \pm 1.99d	0.88 \pm 0.19b
	BR0.01+M	13.90 \pm 1.85c	18.38 \pm 2.85b	0.92 \pm 0.31b
	BR0.1+M	15.53 \pm 1.08bc	19.62 \pm 2.04b	1.38 \pm 0.27a
	BR1+M	17.33 \pm 0.23ab	18.63 \pm 0.77b	1.56 \pm 0.14a
	BR2+M	14.27 \pm 1.72c	13.68 \pm 3.15c	0.78 \pm 0.03b

Data are the mean \pm standard error (n = 3). For each cultivar, different letters in each column indicate significant differences at P = 0.05 as analyzed by the Duncan's multiple range tests.

CK: Control; M: ME+IMS alone; BR0.01+M: 0.01 mg L⁻¹ brassinolide and ME+IMS; BR0.1 + M: 0.1 mg L⁻¹ brassinolide and ME+IMS; BR1+M: 1.0 mg L⁻¹ brassinolide and ME+IMS; BR2+M: 2.0 mg L⁻¹ brassinolide and ME+IMS.

Table 2. Effect of seed presoaking with different concentrations of brassinolide on photosynthetic pigment content in leaves of two foxtail millet cultivars under mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) treatment.

Cultivars	Treatment	Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll
		mg g ⁻¹ FM			
Zhangza 5	CK	0.81a	0.24a	0.26a	1.05a
	M	0.56b	0.13c	0.18b	0.69b
	BR0.01 + M	0.56b	0.14c	0.19b	0.70b
	BR0.1 + M	0.62ab	0.15bc	0.21ab	0.78ab
	BR1 + M	0.81a	0.21ab	0.26a	1.01a
	BR2 + M	0.75ab	0.17bc	0.23ab	0.93ab
Jingu 21	CK	0.91a	0.28a	0.31a	1.19a
	M	0.66a	0.15b	0.20a	0.81ab
	BR0.01 + M	0.68a	0.16b	0.21a	0.84ab
	BR0.1 + M	0.71a	0.17b	0.23a	0.89ab
	BR1 + M	0.90a	0.22ab	0.28a	1.12ab
	BR2 + M	0.61a	0.16b	0.20a	0.77b

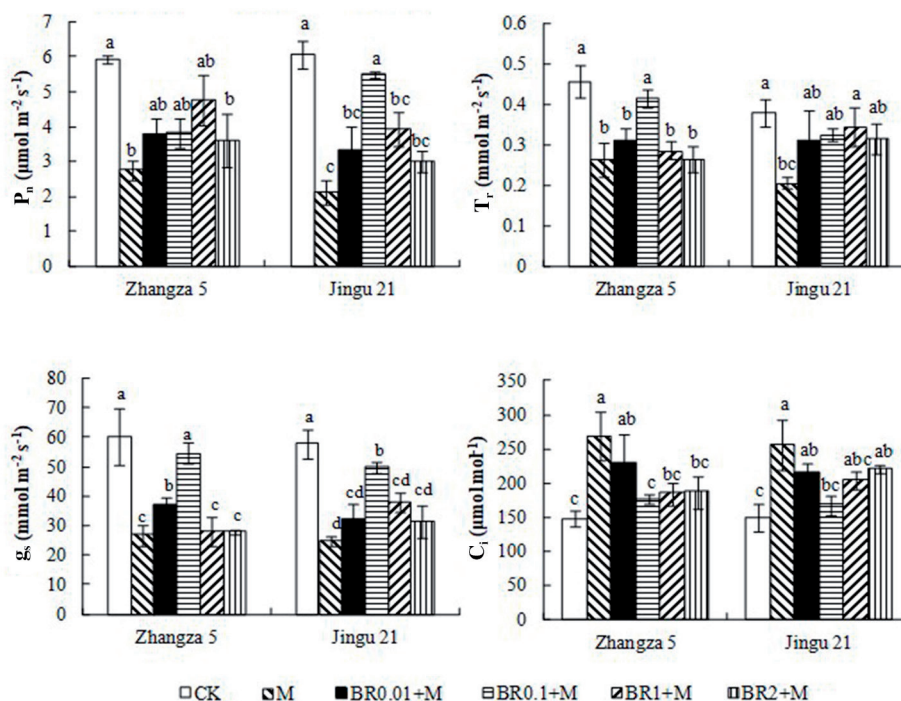
Data are the mean (n = 3). For each cultivar, different letters in each column indicate significant differences at P = 0.05 as analyzed by the Duncan's multiple range tests.

FM: Fresh mass; CK: control; M: ME+IMS alone; BR0.01+M: 0.01 mg L⁻¹ brassinolide and ME+IMS; BR0.1+M: 0.1 mg L⁻¹ brassinolide and ME+IMS; BR1+M: 1.0 mg L⁻¹ brassinolide and ME+IMS; BR2+M: 2.0 mg L⁻¹ brassinolide and ME+IMS.

Photosynthetic gas exchange parameters

Under M treatment, photosynthetic parameters P_n , T_r , and G_s all decreased significantly compared with the control treatment, while C_i exhibited an opposite trend in both cultivars. After brassinolide treatment, P_n , T_r , and G_s all increased compared with M treatment in both cultivars (Figure 1). Under BR1+M compared with M treatment, an increase in P_n of 72.5% was observed in 'Zhangza 5', although the difference was nonsignificant. Meanwhile, in 'Jingu 21', nonsignificant

Figure 1. Effect of seed presoaking with different concentrations of brassinolide on gas exchange parameters in leaves of two foxtail millet cultivars under mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) treatment.



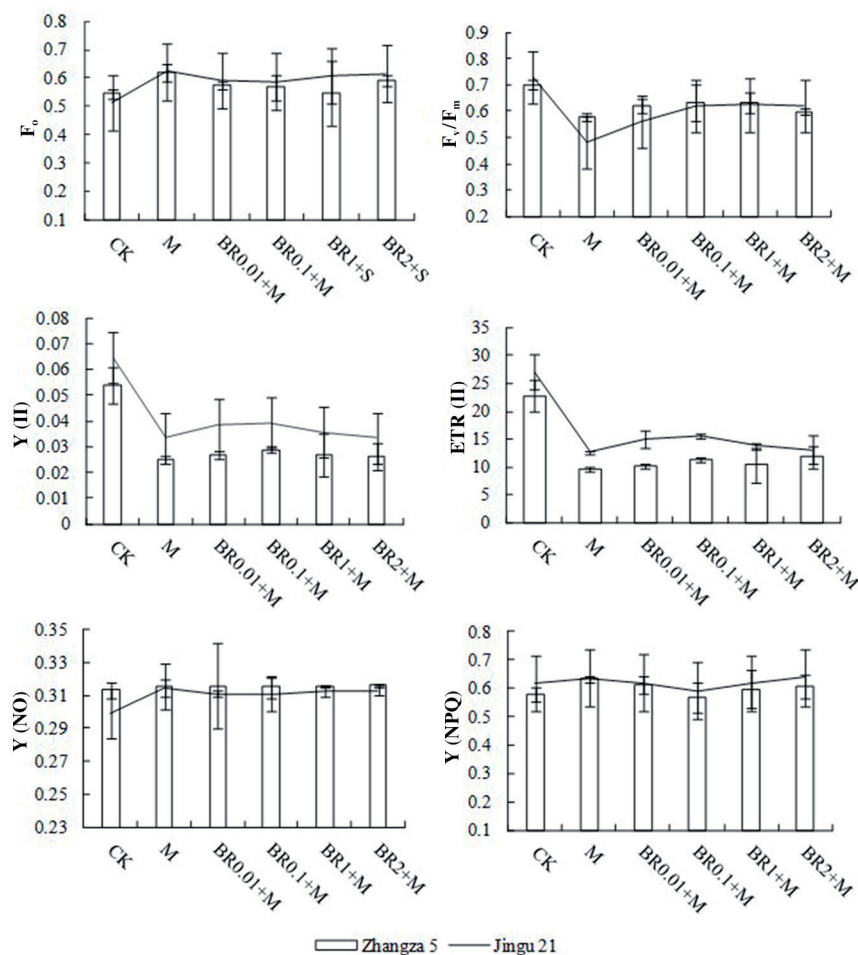
Bars with the same letters above are not significantly different at the 0.05 probability level. Vertical bars correspond to standard error. P_n : Net photosynthetic rate; T_r : transpiration rate; G_s : stomatal conductance; C_i : intercellular CO₂ concentration; CK: control; M: ME+IMS alone; BR0.01+M: 0.01 mg L⁻¹ brassinolide and ME+IMS; BR0.1+M: 0.1 mg L⁻¹ brassinolide and ME+IMS; BR1+M: 1.0 mg L⁻¹ brassinolide and ME+IMS; BR2+M: 2.0 mg L⁻¹ brassinolide and ME+IMS.

increase in P_n was observed until brassinolide concentrations reached 0.1 mg L^{-1} , at which point an increase of 158.5% was observed. Furthermore, significant increases in g_s of 103.1% and 100.4% were observed under BR0.1+M in ‘Zhangza 5’ and ‘Jingu 21’, respectively. The largest T_r in ‘Zhangza 5’ was obtained under BR0.1+M, while in ‘Jingu 21’ the maximum was observed under BR1+M. In both cultivars C_i values were much smaller under BR0.1+M treatment than M treatment.

Chlorophyll fluorescence parameters

The M treatment improved the F_o in both cultivars slightly but not significantly by 14.8% (‘Zhangza 5’) and 21.6% (‘Jingu 21’) compared with control treatment. In contrast, F_v/F_m , $Y(II)$, and $ETR(II)$ decreased by 17.1%, 53.7% and 57.9% in ‘Zhangza 5’, and by 34.2%, 49.2%, and 53.1% in ‘Jingu 21’, respectively, after M treatment (Figure 2). In both cultivars, F_o first decreased then increased to differing degrees with increasing concentrations of brassinolide. Under BR0.1+M treatment, F_v/F_m , $Y(II)$, and $ETR(II)$ increased by 8.6%, 16.0%, and 17.8%, and by 29.2%, 18.2%, and 23.1% in ‘Zhangza 5’ and ‘Jingu 21’, respectively, compared with M treatment. The lowest $Y(NO)$ and $Y(NPQ)$ values in both cultivars were observed under BR0.1+M.

Figure 2. Effect of seed presoaking with different concentrations of brassinolide on chlorophyll fluorescence parameters in leaves of two foxtail millet cultivars under mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) treatment.



Vertical bars correspond to standard error.

F_o : Initial fluorescence; F_v/F_m : photosystem II (PSII) maximum quantum yield; $Y(II)$: PSII effective quantum yield; $ETR(II)$: PSII electron transport rate; $Y(NO)$: quantum yield of non-regulated energy dissipation in PSII; $Y(NPQ)$: quantum yield of regulated energy dissipation in PSII; CK: control; M: ME+IMS alone; BR0.01+M: 0.01 mg L^{-1} brassinolide and ME+IMS; BR0.1+M: 0.1 mg L^{-1} brassinolide and ME+IMS; BR1+M: 1.0 mg L^{-1} brassinolide and ME+IMS; BR2+M: 2.0 mg L^{-1} brassinolide and ME+IMS.

P₇₀₀ parameters

Changes in Y(I), ETR(I), P_m, and Y(ND) were consistent under M treatment, decreasing in both cultivars compared with control treatment. Decreases of 26.5%, 67.0%, 57.9% and 5.0%, and 15.3%, 48.0%, 38.5%, and 3.7% were observed in 'Zhangza 5' and 'Jingu 21', respectively, while an opposite trend in Y(NA) was observed (Table 3). Brassinolide treatment induced an increase in Y(I), ETR(I), P_m, and Y(ND) in both cultivars, with values increasing then decreasing with increasing concentrations of brassinolide. Compared with M treatment, BR0.1+M improved Y(I), ETR(I), P_m, and Y(ND) by 16.7%, 38.7%, 51.2%, and 5.3% in 'Zhangza 5', respectively, while in 'Jingu 21', Y(I), ETR(I), and Y(ND) increased by 7.0%, 21.9% and 2.9% under BR0.1+M treatment, respectively. Under BR1+M treatment, P_m increased by 34.3% in 'Jingu 21', compared with M treatment. In contrast, significant decreases in Y(NA) of 57.1 and 43.5% were observed in 'Zhangza 5' and 'Jingu 21' under BR0.1+M compared with M treatment, respectively.

DISCUSSION

In the present study, the application of ME+IMS at 2.81+0.56 g ai ha⁻¹ inhibited the growth of foxtail millet seedlings in pots, as indicated by a reduction in plant height, leaf area, and fresh mass in both 'Zhangza 5' and 'Jingu 21' (Table 1). This result was in agreement with the previous study by Yuan et al. (2017), in which ME+IMS was found to be unsafe in foxtail millet seedlings. However, it was also reported that prespraying with 0.1 mg L⁻¹ brassinolide could alleviate the phytotoxicity of ME+IMS in foxtail millet. Similarly, in this study, growth inhibition of plant height, leaf area, and fresh mass by ME+IMS was alleviated in both cultivars in a concentration-dependent manner after soaking seeds in brassinolide. Notably, seed soaking in 0.1-1.0 mg L⁻¹ brassinolide resulted in significant recovery of seedling growth under ME+IMS stress (Table 1), possibly due to the ability of brassinolide to modulate cell division and elongation (Zhiponova et al., 2013). Similarly, Sharma et al. (2015) reported an increase in growth parameters in rice seedlings under salt and pesticide stress following exogenous application of brassinolide.

The content of photosynthetic pigment contents is an important indicator of plant photosynthesis (Yuan et al., 2013). In the present study, ME+IMS treatment induced remarkable reductions in all photosynthetic pigment contents in the foxtail millet seedlings (Table 2). This was possibly due to chloroplast degradation, the thylakoid stacking level, chlorophyll oxidation, and an increase in chlorophyllase (Roca and Mínguez-Mosquera, 2003; Harpaz-Saad et al., 2007; Tamary et al., 2019). Moreover, brassinolide was found to maintain the chloroplast integrity and prevent the loss of photosynthetic pigments either by activating or inducing the synthesis of enzymes involved in chlorophyll biosynthesis (Siddiqui et al., 2018). In this study, seed soaking in 1 mg L⁻¹ brassinolide markedly increased the chlorophyll and carotenoid contents

Table 3. Effect of seed presoaking with different concentrations of brassinolide on P₇₀₀ parameters in leaves of two foxtail millet cultivars under mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) treatment.

Cultivars	Treatment	Y(I)	ETR(I)	P _m	Y(ND)	Y(NA)
Zhangza 5	CK	0.098a	71.2a	0.515a	0.874a	0.028c
	M	0.072c	23.5d	0.217b	0.830c	0.098a
	BR0.01+M	0.079bc	27.4cd	0.318b	0.855ab	0.066b
	BR0.1+M	0.084b	32.6b	0.328b	0.874a	0.042c
	BR1+M	0.078bc	30.4bc	0.249b	0.839bc	0.083a
	BR2+M	0.078bc	30.6bc	0.242b	0.837bc	0.085a
Jingu 21	CK	0.118a	69.4a	0.403a	0.862a	0.020c
	M	0.100b	36.1b	0.248b	0.830c	0.069a
	BR0.01+M	0.105b	41.8b	0.278b	0.851b	0.044b
	BR0.1+M	0.107b	44.0b	0.281b	0.854ab	0.039b
	BR1+M	0.106b	40.8b	0.333ab	0.848b	0.046b
	BR2+M	0.105b	37.8b	0.310ab	0.848b	0.046b

Data are the mean (n = 3). For each cultivar, different letters in each column indicate significant differences at P = 0.05 as analyzed by the Duncan's multiple range tests.

Y(I): Photosystem I (PSI) photochemical quantum yield; ETR(I): PSI electron transport rate; P_m: maximal P₇₀₀ change; Y(ND): quantum yield of non-photochemical energy dissipation due to donor side limitation in PSI; Y(NA): quantum yield of non-photochemical energy dissipation due to acceptor side limitation in PSI; CK: control; M: ME+IMS alone; BR0.01+M: 0.01 mg L⁻¹ brassinolide and ME+IMS; BR0.1+M: 0.1 mg L⁻¹ brassinolide and ME+IMS; BR1+M: 1.0 mg L⁻¹ brassinolide and ME+IMS; BR2 + M: 2.0 mg L⁻¹ brassinolide and ME+IMS.

of the foxtail millet seedling leaves under ME+IMS stress (Table 2). Recovery of the chlorophyll content following application of brassinolide has been reported in wheat (Dehghan et al., 2020). Carotenoids bound to discrete pigment-protein complexes are distributed around chlorophylls where they not only absorb solar energy and transfer it to the chlorophylls, but also protect photosynthetic organelles from photoinduced damage (Polívka and Frank, 2010; Xue et al., 2021). This may, to some extent, explain the similar changes in carotenoid and total chlorophyll contents in the leaves of foxtail millet seedlings treated with brassinolide.

Photosynthesis is the basis of crop growth, contributing to more than 90% of crop yield (Makino, 2011). In the present study, P_n decreased considerably, while C_i increased markedly in foxtail millet seedlings showing ME+IMS toxicity (Figure 1). These observations suggest that non-stomatal factors limit photosynthesis in foxtail millet (Zhang et al., 2020a). Moreover, an increase in P_n and decrease in C_i were observed after seed soaking in 0.1-1.0 mg L⁻¹ brassinolide (Figure 1). Brassinolide treatment also relieved non-stomatal limitations to some degree, a possible cause of the increase in P_n under ME+IMS toxicity. The results of the present study are in agreement with several previous studies, whereby brassinolide application was found to enhance photosynthesis in maize, and tung tree seedlings under normal and stress conditions (Sun et al., 2020; Zhang et al., 2020b).

Chlorophyll fluorescence and P_{700} absorbance parameters are more sensitive to herbicide stress than the photosynthetic pigment content and other growth parameters (Zhang et al., 2015). In the absence of actinic light, F_o represents the fluorescence yield of PSII, which indicates full openness of the leaf to light (Maxwell and Johnson, 2000), and the increase in F_o indicates the injury of PSII (Qiu et al., 2013). In the present study, F_o values in the foxtail millet seedlings were largest following treatment with ME+IMS, but decreased after presoaking of seeds with brassinolide (Figure 2). These findings suggest that ME+IMS directly damages PSII in the cotyledons of foxtail millet seedlings, with brassinolide treatment easing damage to some extent. In addition, the F_v/F_m represent the conversion efficiency of primary light energy, a low F_v/F_m ratio indicates the injury of PSII reaction center or stress in plants (Qiu et al., 2013). In this study, the F_v/F_m ratio reduced markedly following ME+IMS treatment, suggesting stress on the photosystem. Interestingly, however, seed soaking with brassinolide increased the F_v/F_m ratio (Figure 2). Meanwhile, $Y(II)$ and $Y(I)$ represent the actual light energy conversion efficiency of PSII and PSI, respectively, while $ETR(II)$ and $ETR(I)$ indicates the electron transport rate of PSII and PSI (Yuan et al., 2017). Higher photochemical quantum efficiency and electron transfer are conducive with the utilization and transformation of light energy by plants. In this study, increases in $Y(II)$, $Y(I)$, $ETR(II)$, and $ETR(I)$ values were observed following treatment with 0.1 mg L⁻¹ brassinolide relative to seedlings under ME+IMS stress alone (Figure 2; Table 3). This result suggests that seed soaking with brassinolide stimulates photosystem opening, in turn increasing the quantum efficiency and electron transport rate in foxtail millet. Clearly, our results from the present study are consistent with the other early reports (Lv et al., 2020; Zhang et al., 2020b).

In this study, ME+IMS treatment increased the $Y(NPQ)$ and $Y(NO)$ values in foxtail millet seedlings compared with control treatment, while seed soaking with brassinolide resulted in lower values compared with ME+IMS alone (Figure 2). A high $Y(NPQ)$ value is used as an indicator of PSII protection, representing the dissipation of excess absorbed light energy into heat, while a high $Y(NO)$ value represents the degree of inhibition of photosynthesis (Fortunato et al., 2018; Xue et al., 2021). The results of this study therefore suggest that brassinolide treatment prevents inhibition by regulating the distribution of energy in PSII as well as increasing the photoprotection capability of foxtail millet. Meanwhile, $Y(ND)$ is the important index of photo-protection in PSI, which indicates the state of electron donors in PSI, as affected by the transmembrane proton gradient. In contrast, $Y(NA)$ is the important index of photo-damage in PSI, which indicates the state of electron acceptors in PSI, as affected by dark adaptation and the level of damage to CO₂ fixation (Yuan et al., 2013).

In the present study, $Y(ND)$ decreased rapidly due to ME+IMS toxicity, while $Y(NA)$ increased (Table 3), suggesting that ME+IMS aggravates PSI damage in the leaves of foxtail millet. Furthermore, an increase in $Y(ND)$ and decrease in $Y(NA)$ values was observed following 0.1 mg L⁻¹ brassinolide treatment compared with ME+IMS treatment alone (Table 3). These findings suggest that presoaking seeds with brassinolide relieved damage to PSI in the foxtail millet leaves, increasing the transmembrane proton gradient, enhancing CO₂ fixation, and unblocking the dark reaction.

Overall, the analyses of chlorophyll fluorescence parameters in foxtail millet suggest that photoinhibition of PSII and PS caused by ME+IMS stress is weakened by presoaking seeds with 0.1-1.0 mg L⁻¹ brassinolide. The mitigating effects of pre-sowing seed treatment with brassinolide on photoinhibition of PSII and PSI is thought to occur partly due to the increase in photosynthetic pigment content, since the accumulation of photosynthetic pigments and enhanced electron transfer rate were able to balance the decrease in photoprotection and photoinhibition (Xue et al., 2021).

CONCLUSIONS

Presoaking of seeds with 0.1-1.0 mg L⁻¹ brassinolide maintained a relatively high plant height, leaf area, and fresh mass in two foxtail millet cultivars under mesosulfuron-methyl + iodosulfuron-methyl-sodium (ME+IMS) stress. Brassinolide treatment also increased chlorophyll and carotenoid contents, photosynthetic rate, transpiration rate, and stomatal conductance, while weakening the inhibitory effects of ME+IMS on chlorophyll fluorescence of PSII and PSI. Brassinolide treatment at 0.1 and 1.0 mg L⁻¹ exhibited a better effect on relieving ME+IMS stress in 'Zhangza 5' and 'Jingu 21', respectively. Overall, the findings suggest that pre-sowing seed treatment with 0.1-1.0 mg L⁻¹ brassinolide improved plant growth and photosynthetic performance, in turn enhancing the resistance of foxtail millet seedlings to ME+IMS stress.

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