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Original article

# The Growth, physiological and biochemical response of foxtail millet to atrazine herbicide

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# ABSTRACT

Foxtail millet (*Pennisetum glaucum* L.) is a vital crop that is planted as food and fodder crop around the globe. There is only limited information is present for abiotic stresses on the physiological responses to atrazine. A field experiment was conducted to investigate the effects of different atrazine dosages on the growth, fluorescence and physiological parameters i.e., malonaldehyde (MDA) and reactive oxygen species (ROS) (H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>) in the leaves to know the extent of atrazine on oxidative damage of foxtail millet. Our experiment consisted of 0, 2.5, 12.5, 22.5 and 32.5 (mg/kg) of labeled atrazine doses on 2 fox-taill millet varieties. High doses of atrazine significantly enhanced ROS and MDA synthesis in the plant leaves. Enzymes activities like ascorbate peroxidase (APX) and peroxidase (POD) activities enhanced, while catalase (CAD) and superoxide dismutase (SOD) activities reduced with increasing atrazine concentrations. Finally atrazine doses at 32.5 mg/kg reduced chlorophyll contents, while chlorophyll (a/b) ratio also enhanced. Biomass, plant height, chlorophyll fluorescence parameters, minimal and maximal fluorescence (Fo, Fm), maximum and actual quantum yield, photochemical quenching coefficient, and electron transport rate are decreased with increasing atrazine doses.

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#### 1. Introduction

Foxtail millet (Setaria italica L.) is a warm season annual grass crop and has been grown in Africa and Indian subcontinent since prehistoric times. Because of its tolerance to difficult growing conditions, it can be grown in areas where other cereal crops, such as maize or wheat, would not survive. Foxtail millet is a cereal crop

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and it has bundle of energy, protein, iron and zinc and lot of antioxidants (Gopalan et al., 2006; Nambiar et al., 2011). For nutritional purpose, 100 g of millet contains, 351 calories, 11.2 g of protein, 4 g of total fat, 63.2 g of carbohydrate. Millet is used to control tumor, blood pressure and and plasma-low-density protein, cholesterol levels and to control diabetes (Nambiar et al., 2012). Millet contains high amount of energy, protein and fat (Gopalan et al., 2006) iron, zinc and has high amount of antioxidants (Nambiar et al., 2011). These dietary nutrients are very useful for healthy and fit for life. It is also important to control diseases like tumor treatment, blood pressure, cholesterol level and diabetes in the body (Nambiar et al., 2012).

Atrazine is used to control broadleaf and grassy weeds (Erinle et al. 2018). Tomer and Singh (1973) and Panchal and Sastry (1974) reported that, atrazine used before weed emergence enhanced millet yield by 28% and 100%. Millet seedlings exposure to atrazine reduces germination, shoot and biomass production (Dan et al., 2010; Burhan and Shaukat 2000) which is main reason for caused plant cell death (Singh et al., 2004). Atrazine herbicide is

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*Abbreviations:* Malonaldehyde, MDA; Reactive oxygen species, ROS; Ascorbate peroxidase, APX, peroxidase, POD; thiobarbituric acid, TBA; Superoxide dismutases, SOD; Glutathione reductase, GR; Bensulfuron-methyl, BSM.

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major cause of the reduction of photosynthesis, by reducing the photosystem II (Bai et al., 2015). The reduction of PSII electron transport inhibited the conversion of chlorophyll absorbed light energy into electro-chemical energy which ultimately produces triplet chlorophyll and singlet oxygen (Perez-Jones et al., 2009). The fast production of these reactive radicals, are called as the ROS, which ultimately effected APX, POD, CAT production in PSII, which ultimately destroyed proteins, lipids and pigments (Dan Hess 2000; Zhu et al. 2009).

Mainly, physiological mechanisms such as photosynthesis process are reduced by damaging stomatal and non-stomatal openings (Rahnama et al., 2010). In this research, antioxidant enzymes for example SOD, POD, APX, MDA and ROS and their physiological mechanism were studied under different atrazine concentrations. The present study was designed to investigate the physiological and biochemical behavior of foxtail millet varieties to atrazine, and to estimate the optimize and suitable dose of atrazine for controlling pollution in foxtail millet.

## 2. Materials and methods

#### 2.1. Experimental design

Atrazine (10%, WP) was provided by Dengfeng Jinbo Pesticide Chemical Co., Ltd. The seeds Zhangza 10 (foxtail millet variety) were supplied by the Zhangjiakou Academy of Agricultural Sciences. The seeds of Jingu 21 were provided by Shanxi Academy of Agricultural Sciences. Experimental study was conducted during 2019 at the farm of Shanxi Agricultural University, China. The experimental site has a temperate continental climate. The soil is loamy containing 20.08 g kg<sup>-1</sup> organic matter, 37.33 mg kg<sup>-1</sup> alkaline N, 24.3 mg kg<sup>-1</sup> available P, and 90.01 mg kg<sup>-1</sup> available K at 0-20 cm soil layer. The experiment consist of randomized complete block design with three replicates. After germination at three-leaf to five-leaf stage, the plants were treated with 0 control (CK), 2.5 (T1), 12.5 (T2), 22.5 (T3) and 32.5 (T4) mg/kg atrazine. The application was made with a laboratory pot-sprayer equipped with a nozzle, adjuster to deliver 450 L/ha. The data on agronomic characters, physiological parameters, and chlorophyll fluorescence of foxtail millet seedlings were recorded after 10 and 20 days. The yield and quality parameters were measured at maturity stage.

# 2.2. Measurements

#### 2.2.1. Plant height

The plant height, length and width of leaves were measured with meter rod. The Stem thickness measured with vernier caliper. After harvesting, measure the ear length, ear weight, ear thickness, ear grain weight and other indicators with a ruler, vernier caliper, and a ten-thousandth analytical balance (Mettler-Toledo, LLC. Shanghai, China).

#### 2.2.2. Physiological parameters

Physiological parameters like photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*Gs*) and intercellular CO<sub>2</sub> concentrations (*Ci*) were measured by CI-340 portable photosynthesis system (CID Bio-Science, Inc., USA) during day time 10:00 to 11:00 am. The maximum photochemical efficiency (*Fv*/*Fm*), apparent electron transport rate (ETR) and photochemical quenching coefficient (*qP*) and non-photochemical quenching coefficient (*qN*) were measured by the miniaturized pulse-amplitude modulated fluorescence analyzer (Mini-PAM, Walz, Effeltrich, Germany) from 8:30 to 10:00 pm (according to Guo et al., 2018), briefly described here. The Chlorophyll *a* content, Chlorophyll *b* content,

Total Chlorophyll content and caroteniad contents were determined according to (Yuan et al., 2017).

#### 2.2.3. Enzymatic parameters

MDA was determined by the thiobarbituric acid (TBA) test according to (Gao, 2006). The activity of APX was determined according to the method of (Yoshiyuk, 1981). Fresh foxtail millet leaf (0.1 g) was homogenized in 2 mL 0.5 mol/L phosphate buffer (PH 7.0), and centrifuged at 10000  $\times$  g for 10 min at 4 °C. The glutathione reductase (GR) activity was carried out according to (Halliwell et al., 1978). Fresh foxtail millet leaf (0.1 g) was weighed into an ice-cooled mortar, grinded in an ice bath with 2 mL 1 mol/L Tris-HCl (PH 7.5), and centrifuged at 13000  $\times$  g for 20 min at 4 °C according to method described by Guo et al., (2018).

The (AsA/DHA) was determined according to Jiang (2001) briefly described here. Fresh leaf (0.1 g) was mixed in 2 mL of 5% chilled sulfosalicylic acid in an ice bath and centrifuged at 10,000  $r min^{-1}$  for 15 min at 4 °C. This method determined the total ascorbic acid content and to measure the amount of AsA used to replace DTT and n-ethylmaleimide. Nagalakshmi (2001) method was used to estimate (GSH/GSSG) briefly described here. This is standardized method which is used to estimate GSSG and the total glutathione used in isopycnic distilled water to replace 2-vinylpyridine. Fresh foxtail millet leaf (0.1 g) was weighed into an ice-cooled mortar, grinded in an ice bath with 2 mL 5% chilled sulfosalicylic acid in an ice bath and centrifuged at 10 000 r min  $^{-1}$  for 15 min at 4 °C. A 200-µL aliquot of the supernatant was removed and neutralized by the addition of 24  $\mu$ L 1.84 mol L<sup>-1</sup> triethanolamine, 50  $\mu$ L 2vinylpyridine and 5% chilled sulfosalicylic acid for 60 min at 25 °C for GSSG reductase to mask GSH via derivatization and to allow for the determination of GSSG alone. The above reaction was mixed with 2.7 mL 0.05 mol L<sup>-1</sup>Na-phosphate buffer (pH 7.5, containing 2.5 mmol L<sup>-1</sup> EDTA), 20 µL 0.01 mmol L<sup>-1</sup> nicotinamide adenine dinucleotide phosphate (NADPH) and 80 µL 12.5 mmol L<sup>-1</sup> 5,5-dithiobis- (2-nitrobenzoic acid) DTNB for 10 min at 25 °C. A 20- $\mu$ L aliquot of glutathione reductase (50 U mL<sup>-1</sup>) was added and the change in absorbance at 412 nm was monitored for 3 min. This method was used to measure GSSG and the total glutathione used in isopycnic distilled water to replace 2-vinylpyridine.

# 2.3. Statistical analysis

For statistical analysis of the data Microsoft Office Excel 2010 and data processing system (DPS 7.05) were used. Duncan's test was used to estimate significant differences among the treatments. We used P = 0.05 as the statistical significance threshold.

# 3. Result

#### 3.1. Effects of atrazine on gas exchange parameters of foxtail millet

Under atrazine stress, the plant height, leaf area and stem diameter of Jingu 21 and Zhangzagu 10 significantly decreased to varying degrees, and there was a certain dose effect (Table 1). Atrazine treatments from T1 to T4 significantly decreased the agronomic traits of foxtail millet seedlings after 10 and 20 days. After 10 days of atrazine application, the plant height, leaf area and stem thickness of Jingu 21 and Zhangzagu 10 were significantly decreased by 39.71%, 31.40%, 11.49%, 41.22%, 30.34%, 11.32%, respectively; while, decreased by 29.43%, 17.39%, 13.78%, 38.65%, 33.08%, 26.17% after 20 days. Therefore, at the initial stage of application, the recommended dose of atrazine had severe phytotoxicity to both Jingu 21 and Zhangzagu 10, but the phytotoxicity gradually ceased over time, and the relief capacity of Jingu 21 was slightly stronger than Zhangzagu 10.

#### Table 1

Effects of atrazine on agronomic traits of foxtail millet.

| Varieties    | Treatments | Plant height(cm) |                 | Leaf area(cm <sup>2</sup> ) |                | Stem diameter(mm) |              |
|--------------|------------|------------------|-----------------|-----------------------------|----------------|-------------------|--------------|
|              |            | 10d              | 20d             | 10d                         | 20d            | 10d               | 20d          |
| Jingu 21     | СК         | 77.19 ± 5.37a    | 106.88 ± 3.9a   | 108.16 ± 7.16a              | 127.33 ± 3.70a | 9.55 ± 0.8a       | 10.78 ± 1.6a |
|              | T1         | 49.77 ± 4.20b    | 88.42 ± 9.8b    | 78.05 ± 3.76b               | 112.75 ± 3.50b | 7.33 ± 0.7b       | 9.29 ± 0.9b  |
|              | T2         | 46.70 ± 3.90b    | 76.50 ± 14.1bc  | 68.73 ± 3.31b               | 108.16 ± 8.02b | 7.5 ± 0.9b        | 9.39 ± 0.8b  |
|              | T3         | 45.83 ± 3.15b    | 67.42 ± 16.9c   | 58.66 ± 5.96c               | 99.98 ± 6.31c  | 7.25 ± 0.4b       | 9.14 ± 0.8b  |
|              | T4         | 41.07 ± 3.40b    | 67.94 ± 18.64c  | 58.52 ± 5.02c               | 90.47 ± 7.18d  | 6.97 ± 0.7b       | 8.85 ± 0.7b  |
| Zhangzagu 10 | СК         | 72.26 ± 5.40a    | 98.49 ± 10.20a  | 92.78 ± 4.82a               | 115.38 ± 4.11a | $7.40 \pm 0.1a$   | 11.47 ± 0.8a |
|              | T1         | 45.96 ± 5.41b    | 62.90 ± 15.27b  | 67.95 ± 4.23b               | 80.87 ± 4.24b  | $8.24 \pm 0.5a$   | 8.55 ± 1.4ab |
|              | T2         | 43.19 ± 4.52bc   | 58.44 ± 18.5b   | 65.59 ± 4.17bc              | 75.54 ± 3.17c  | 7.33 ± 0.9b       | 8.38 ± 1.4b  |
|              | T3         | 41.88 ± 3.30bc   | 51.48 ± 11.61bc | 60.52 ± 3.67c               | 70.65 ± 3.13d  | 6.41 ± 0.9b       | 8.33 ± 0.5b  |
|              | T4         | 40.33 ± 3.49c    | 44.69 ± 7.86c   | 50.30 ± 4.83d               | 62.13 ± 1.42e  | 5.51 ± 0.9c       | 7.20 ± 1.4c  |

Different letters indicate significant differences at P < 0.05 in the same column and variety. The same as below.

## 3.2. Effects of atrazine on photosynthetic pigment of foxtail millet

The contents of photosynthetic pigment in leaves of foxtail millet were reduced significantly with increasing atrazine doses (see in table 2). Apart from the T1 treatment, the total chlorophyll content of foxtail millet in all other treatments was significantly decreased than that of CK after 10 and 20 days, but the content of carotenoid had no significant difference with CK (control). After 10 days of atrazine application, the content of chlorophyll a content in Jingu 21 and Zhangzagu 10 was significantly decreased by 12.96%, 17.70%, 18.64% and 16.52%, 19.17%, 18.50% from T2 to T4 treatment, respectively; After 20 days of atrazine application, the content of chlorophyll *a* content returns to CK level with T2 treatment, but T3 and T4 treatments were still significantly lower than CK, and were significantly reduced by 10.47%, 13.95%, 13.92%, and 15.19%, respectively. The content of chlorophyll *b* content in Iingu 21 had no significant difference with CK under the recommended dosage after 10 days, but that in Zhangzagu 10 was significantly decreased. Treatments with dosages over T1, the content of chlorophyll *b* content showed significant differences with the control after 20 days of application.

# 3.3. Effects of atrazine on gas exchange parameters of foxtail millet

The *Pn*, *Tr* and *Gs* in foxtail millet leaves were decreased with different atrazine dosages treatment; however, the change tendency of *Ci* is opposite (Fig. 1). After 7 days of recommended dose of atrazine treatment application, the *Pn*, *Tr* and *Gs* of Jingu 21 was

Effects of atrazine on photosynthetic parameters of foxtail millet.

decreased by 13.99%, 12.66% and 11.23% compared with control, but the *Ci* of Jingu 21 had no significant difference; the *Tr* and *Gs* of Zhangzagu 10 was decreased by 5.08% and 9.92, the *Ci* increased by 8.06%, but the *Pn* of Zhangzagu 10 had no significant difference with CK. After 15 days of herbicide treatment, the gas exchange parameters of foxtail millet seedlings showed varying degrees of recovery, the *Pn* and *Tr* of Jingu 21 gradually decreased to the control level, and the *Pn* and *Ci* of Zhangzagu 10 recovered to no significant difference from CK.

After high-dose atrazine treatment (T3, T4), the gas exchange parameters of foxtail millet seedlings had always significant difference compared with CK. The *Pn*, *Tr* and *Gs* of the millet leaves were the lowest and *Ci* was the highest after T4 treatment. After 7 and 15 days of treatment, the *Pn*, *Tr* and *Gs* of Jingu 21 was significantly decreased by 23.37%, 15.75%, 7.86% and 27.03%, 13.33%, 8.85%, *Ci* was significantly increased by 14.47% and 13.88%; the *Pn*, *Tr* and *Gs* of Zhangzagu 10 was significantly decreased by 43.34%, 23.20%, 15.94% and 22.40%, 28.90%, 13.32%, *Ci* was significantly increased by 12.32% and 10.25%, separately. According to our results, the effect of atrazine herbicide on the gas exchange parameters of Zhangzagu 10 was higher than that of Jingu 21 and fertility process was not affected.

# 3.4. Effects of atrazine on chlorophyll fluorescence of foxtail millet

As shown in Table 3, with the increase of the dosage of atrazien, the Fv/Fm, ETR and qP of Jingu 21 and Zhangzagu 10 showed a decreasing trend, while qN showed an increasing trend. After 10

| Varieties    | Application<br>of days(d) | Treatments | Chlorophyll <i>a</i><br>content (mg•g <sup>-1</sup> FW) | Chlorophyll <i>b</i> content<br>(mg•g <sup>-1</sup> FW) | Carotenoid content<br>(mg•g <sup>-1</sup> FW) | Chlorophyll content<br>(mg•g <sup>-1</sup> FW) |
|--------------|---------------------------|------------|---|---|---|--|
| Jingu 21     | 10                        | СК         | 1.78 ± 0.03a  | 0.56 ± 0.09a  | 0.31 ± 0.02a                                  | 2.76 ± 0.1a                                    |
| , ,          |                           | T1         | 1.66 ± 0.9ab  | 0.53 ± 0.04a  | 0.29 ± 0.03a                                  | 2.16 ± 0.2b                                    |
|              |                           | T2         | 1.53 ± 0.11bc   | 0.49 ± 0.11a  | 0.28 ± 0.03a                                  | 1.98 ± 0.2bc                                   |
|              |                           | T3         | 1.55 ± 0.07bc   | 0.39 ± 0.01b  | 0.27 ± 0.01a                                  | 1.86 ± 0.0c                                    |
|              |                           | T4         | 1.40 ± 0.15c  | 0.36 ± 0.02b  | 0.27 ± 0.02a                                  | 1.80 ± 0.2c                                    |
|              | 20                        | CK         | 1.73 ± 0.02a  | 0.61 ± 0.04a  | 0.26 ± 0.0a                                   | $2.32 \pm 0.05a$                               |
|              |                           | T1         | 1.64 ± 0.10ab   | 0.55 ± 0.05ab   | 0.26 ± 0.0a                                   | $2.14 \pm 0.09b$                               |
|              |                           | T2         | 1.66 ± 0.08ab   | 0.51 ± 0.06b  | 0.25 ± 0.02a                                  | $2.12 \pm 0.08b$                               |
|              |                           | T3         | 1.54 ± 0.12b  | 0.48 ± 0.04b  | 0.25 ± 0.02a                                  | 2.01 ± 0.1bc                                   |
|              |                           | T4         | 1.50 ± 0.06b  | $0.45 \pm 0.02b$  | 0.26 ± 0.02a                                  | 1.92 ± 0.0c                                    |
| Zhangzagu 10 | 10                        | CK         | 1.87 ± 0.07a  | 0.69 ± 0.08a  | 0.28 ± 0.03a                                  | $2.54 \pm 0.1a$                                |
|              |                           | T1         | 1.72 ± 0.07ab   | 0.54 ± 0.1ab  | 0.31 ± 0.01a                                  | 2.25 ± 0.17b                                   |
|              |                           | T2         | 1.59 ± 0.11bc   | 0.43 ± 0.02bc   | 0.30 ± 0.0a                                   | 2.0 ± 0.08bc                                   |
|              |                           | T3         | 1.53 ± 0.06c  | $0.40 \pm 0.01 bc$                                      | 0.28 ± 0.01a                                  | 1.90 ± 0.9c                                    |
|              |                           | T4         | 1.51 ± 0.11c  | 0.35 ± 0.07c  | 0.29 ± 0.0a                                   | 1.84 ± 0.17c                                   |
|              | 20                        | СК         | 1.56 ± 0.10a  | 0.51 ± 0.02a  | 0.27 ± 0.02a                                  | 2.08 ± 0.10a                                   |
|              |                           | T1         | 1.54 ± 0.05a  | 0.50 ± 0.02ab   | 0.25 ± 0.02a                                  | $2.05 \pm 0.04$ ab                             |
|              |                           | T2         | 1.44 ± 0.11ab   | 0.42 ± 0.04bc   | 0.24 ± 0.03a                                  | 1.84 ± 0.17bc                                  |
|              |                           | T3         | 1.37 ± 0.03ab   | 0.38 ± 0.01c  | 0.24 ± 0.01a                                  | 1.75 ± 0.03c                                   |
|              |                           | T4         | 1.32 ± 0.01b  | 0.36 ± 0.02c  | 0.22 ± 0.01a                                  | 1.68 ± 0.9c                                    |

Different letters indicate significant differences at P < 0.05 in the same column and variety. The same as below.



Fig. 1. Effects of atrazine on gas exchange parameters of foxtail millet seedlings.

# Table 3

Effects of atrazine on chlorophyll fluorescence of foxtail millet.

| Varieties    | Application of days(d) | Treatment | Fv/Fm            | $ETR/(\mu mol \cdot m^{-2} \cdot s^{-1})$ | qP               | qN                |
|--------------|------------------------|-----------|------------------|---|------------------|-------------------|
| Jingu 21     | 10                     | СК        | 0.66 ± 0.05a     | 62.28 ± 3.4a                              | $0.36 \pm 0.02a$ | $0.54 \pm 0.01 b$ |
|              |                        | T1        | 0.60 ± 0.05ab    | 52.64 ± 3.7b                              | 0.33 ± 0.02ab    | 0.56 ± 0.02b      |
|              |                        | T2        | 0.58 ± 0.03b     | 52.26 ± 2.9bc                             | $0.30 \pm 0.03b$ | 0.61 ± 0.03a      |
|              |                        | T3        | $0.56 \pm 0.02b$ | 49.10 ± 1.8bc                             | 0.28 ± 0.04bc    | 0.60 ± 0.05a      |
|              |                        | T4        | 0.55 ± 0.05b     | 44.390 ± 2.6c                             | 0.26 ± 0.01c     | 0.61 ± 0.02a      |
|              | 20                     | CK        | 0.68 ± 0.04a     | 61.90 ± 0.5a                              | 0.38 ± 0.01a     | 0.56 ± 0.01b      |
|              |                        | T1        | 0.65 ± 0.05a     | 57.52 ± 2b                                | 0.39 ± 0.02a     | 0.55 ± 0.04b      |
|              |                        | T2        | 0.63 ± 0.05a     | 55.00 ± 2b                                | 0.34 ± 0.01ab    | 0.67 ± 0.02a      |
|              |                        | T3        | 0.59 ± 0.01b     | 56.93 ± 1b                                | $0.30 \pm 0.01b$ | 0.71 ± 0.04a      |
|              |                        | T4        | 0.57 ± 0.01b     | 52.97 ± 3.0b                              | 0.31 ± 0.02b     | 0.72 ± 0.03a      |
| Zhangzagu 10 | 10                     | CK        | 0.63 ± 0.04a     | 60.13 ± 1a                                | 0.29 ± 0.03a     | 0.55 ± 0.02c      |
|              |                        | T1        | 0.55 ± 0.02ab    | 56.52 ± 3b                                | 0.28 ± 0.03ab    | 0.61 ± 0.03bc     |
|              |                        | T2        | 0.53 ± 0.02ab    | 55.36 ± 2bc                               | 0.29 ± 0.02ab    | 0.61 ± 0.04bc     |
|              |                        | T3        | 0.52 ± 0.03ab    | 53.19 ± 2.2bc                             | 0.27 ± 0.02b     | 0.66 ± 0.01a      |
|              |                        | T4        | 0.51 ± 0.03b     | 49.76 ± 3c                                | 0.25 ± 0.03b     | $0.64 \pm 0.04a$  |
|              | 20                     | CK        | 0.63 ± 0.03a     | 65.36 ± 2a                                | 0.28 ± 0.01a     | 0.61 ± 0.01b      |
|              |                        | T1        | 0.60 ± 0.03a     | 60.52 ± 4ab                               | 0.33 ± 0.03a     | 0.61 ± 0.02b      |
|              |                        | T2        | 0.56 ± 0.01ab    | 58.23 ± 3b                                | 0.19 ± 0.03b     | 0.67 ± 0.03a      |
|              |                        | T3        | 0.52 ± 0.01bc    | 53.56 ± 3b                                | 0.28 ± 0.03a     | 0.68 ± 0.02a      |
|              |                        | T4        | $0.50 \pm 0.01c$ | 56.440 ± 2b                               | $0.20 \pm 0.01b$ | $0.72 \pm 0.04a$  |

Different letters indicate significant differences at P < 0.05 in the same column and variety. The same as below.

days of exposure to atrazine, the *Fv/Fm*, *ETR* and *qP* of Jingu 21 significantly decreased compared with control, while *qN* was significantly increased than that of CK. After exposing the seedlings to T2 for 20 days, the *Fv/Fm* and *qP* recover to the control level, ETR significantly decreased by 11.26%, *qN* significantly increased by 10.85%. The chlorophyll fluorescence characteristics of Jingu 21 were still significantly different from CK after T3 and T4 treatment. The ETR of Zhangzagu 10 was significantly reduced by 9.33% and 11.23%, while *qN* significantly increased by 13.52% and 10.00% compared with control. After the results of recommended dose after 10 and 20 days of application; compared with control, the chlorophyll fluorescence characteristics of foxtail millet seed-lings had always significant difference compared with control after high-dose of atrazine treatment (T3, T4).

# 3.5. Effects of atrazine on MDA contents and SOD, POD, CAT, APX and GR activities of foxtail millet

Increasing dosages of atrazine increased MDA content of Jingu 21 and Zhangzagu 10 (Fig. 2A). Apart from the T1 treatment, the MDA content of foxtail millet in all other treatments was significantly increased compared with control after 7 days. Afte 15 days of exposure to atrazine, the MDA content of Jingu 21 was recovered to the control level, but it was significantly increased by 25.30% and 26.70% at T3 and T4 treatment; the content of MDA in Zhangzagu 10 had no significant difference as compared with control when treated with T2 and T3, but it significantly increased by 27.70% compared with control at the T4 dose.

As shown in Fig. 2B, with the increase of atrazine concentration, the activity of SOD increased and subsequently decreased. The maximum expression levels of SOD activity obtained after treatment with T2 and T3 for 7 days, and the SOD activity of Jingu 21 and Zhangzagu 10 was significantly increased by 12.17% and 10.11%. After 15 days atrazine treatment, the SOD activity of foxtail millet showed various degrees of relief in each treatment, but they were still significantly higher than control treatment in T2, T3 and T4 treatments.

With the increase of the dosage of atrazine, the POD activity of Jingu 21 increased, and Zhangzagu 10 increased and subsequently decreased (Fig. 2-C). Apart from the T1 treatment, the POD activity of foxtail millet in all other treatments was significantly increased compared with control after 7 days. After 15 days of exposure to atrazine, the POD activity of Jingu 21 was recover to the control level at the T2 and T3 dose compared with CK, but that in Zhangzagu 10 was significantly increased by 15.01% and 16.27%, respectively; the POD activity of Jingu 21 was significantly increased by 19.33% compared with control at the T4 dose, but there is no significant difference as compared to control in Zhangzagu 10.

Under atrazine stress, the CAT activity of Jingu 21 and Zhangzagu 10 all decreased to varying degrees (Fig. 2-D). Apart from the T1 treatment, the CAT activity of Jingu 21 in all other treatments was significantly increased by 29.11%, 40.03%, 48.03% and 17.88%, 18.63%, 35.77%, compared with control after 7 and 15 days. The CAT activity of Zhangzagu 10 was highest in T4 treatment after 7 days and T3 treatment after 15 days, which were significantly increased by 33.45% and 24.66% compared with control treatment, respectively.

With the increase of atrazine concentration, the activity of APX increased and subsequently decreased, APX activity reached the highest during T3 treatment (Fig. 2-E). Compared with control, atrazine treatment at the recommended dose increased the APX activity by 22.43% and 28.20% for Jingu 21 and Zhangzagu 10 after 7 days of treatment application, and after 15 days of herbicide application, it recovered to the control level. Under the T3 and T4

atrazine treatment, the APX activity of the foxtail millet is always significantly higher than that of CK, and there was no obvious relief with the progress of the fertility process.

With the increase of the dosage of atrazine, the GR activity of Jingu 21 increased and subsequently decreased, and Zhangzagu 10 increased (Fig. 2-F). After 7 days of application, the GR activity of the foxtail millet was significantly higher than that of control when treated with T2, T3 and T4; after 15 days of the application, the GR activity of Jingu 21 was still significantly higher than that of control while Zhangzagu 10 recovered to the control level under T2 and T3 treatment, and was significantly increased by 10.13% compared with CK under T4 treatment.

## 3.6. Effects of atrazine on antioxidant content of foxtail millet

Different dosages of atrazine have different effects on AsA, DHA, Total AsA content and AsA/DHA ratio of foxtail millet leaves (Table 4). The content of AsA, DHA and total AsA increased with the increase in atrazine dosage. After exposing the seedlings to the recommended dose of atrazine, the AsA, total AsA content and AsA/DHA ratio of Jingu 21 was significantly increased by 41.11%, 18.03% and 63.31%, respectively; after 20 days, AsA content recovered to the control level, while total AsA content and AsA/DHA ratio were still significantly higher than CK. The content of AsA, DHA and total AsA of Zhangzagu 10 were significantly higher than those of control treatment after 10 and 20 days of application, the content of AsA, DHA and total AsA has no obvious relief, the DHA content was not significantly different from that of control treatment when compared with with T1, T2 and T3.

# 3.7. Effects of atrazine on GSH, GSSG, total GSH and GSH/GSSG ratio of foxtail millet

With the increase of atrazine concentration, the GSH and total GSH content of foxtail millet increased, and the GSSG content increased and subsequently decreased (Table 5). Compared with control, the GSH and total GSH content of Jingu 21 significantly increased under T2, T3 and T4 treatment after 10 and 20 days; GSH/GSSG ratio significantly increased by 11.44% under T4 treatment after 7 days, while after 20 days, it recovered to the control level; the GSSG content has no significant difference with CK in all treatments. After 10 days of application, the GSH, GSSG and total GSH content of Zhangzagu 10 significantly increased than CK (control) in all treatments, while the GSH/GSSG ratio significantly decreased by 53.43%, 39.72% and 57.05% under T1, T2 and T3 treatment, respectively; The GSH and total GSH content significantly higher than CK after exposing the seedlings to atrazine for 20 days, the content of GSSG increased significantly compared with CK under T4 treatment and was not significantly different from CK under other treatments, The GSH/GSSG ratio all recovered to the control level.

## 3.8. Effects of atrazine on yield and yield components of foxtail millet

Under atrazine stress, the ear length, ear diameter, spike weight, grain weight, ear yardage, 1000-grains weight, number of ears and yield of Jingu 21 and Zhangzagu 10 decreased significantly (Table 6). Under T1 treatment, the yield of Jingu 21 was significantly reduced 7.50% as compared with CK, while the difference between Zhangzagu 10 and CK was not significant. When treated with T2, T3 and T4, the yield of Jingu 21 and Zhangzagu 10 were significantly reduced by 14.75%, 33.45%, 50.03%, 24.12%, 39.20% and 50.08%, respectively.



Fig. 2. Effects of atrazine on MDA contents and SOD v POD v CAT v APX v GR activities of foxtail millet.

# 4. Discussion

In foxtail millet leaves atrazine has damaged photosynthetic pigments by chemotoxicity and chloroplast dysfunction (Xing et al., 2013). The reason of this is that highest atrazine dose at (50 mg/kg) droped chlorophyll content because chl is known as a sensitive indicator for plant growth (Wei et al., 2011). The main reason of this is also the destruction effect of atrazine on chlorophyll pigment. Decrease in chlorophyll content is due to very high

chlorophyll depletion instead of its slow release. Main discovery of our study is that chl (a/b) ratio enhanced due to atrazine stress. Chloroplasts are major site of protein degradation, which is initiated by ROS (Khanna-Chopra 2012) and 75% cellular nitrogen is located in mesophyll cells (H€ortensteiner and Feller 2002).

Different reports demonstrated that excessive application of post-emergent herbicides like fluroxypyr inhibited plant growth (Guo et al., 2018; 2019). In this experiment, we recorded that atrazine had less effect on plant growth at low doses, but have bad

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#### Table 4

Effects of atrazine on AsA, DHA, total AsA and AsA/DHA ratio of foxtail millet.

| Varieties    | Application of days<br>(d) | Treatment<br>(g·hm <sup>-2</sup> ) | AsA content<br>(µmol·g <sup>-1</sup> FW) | DHA content<br>(µmol·g <sup>-1</sup> FW) | Total AsA Content<br>(μmol·g <sup>-1</sup> FW) | AsA/DHA          |
|--------------|----------------------------|------------------------------------|--|--|--|------------------|
| Jingu 21     | 10                         | СК                                 | 1.11 ± 0.2c                              | 0.77 ± 0.1b                              | 1.82 ± 0.3d                                    | 1.47 ± 0.03b     |
|              |                            | T1                                 | $1.46 \pm 0.03b$                         | $0.44 \pm 0.0c$                          | 1.88 ± 0.1 cd                                  | $3.39 \pm 0.5a$  |
|              |                            | T2                                 | 1.63 ± 0.05ab                            | 0.63 ± 0.2bc                             | 2.27 ± 0.1bc                                   | 2.69 ± 1.0a      |
|              |                            | T3                                 | 1.80 ± 0.21a                             | 0.61 ± 0.0bc                             | 2.44 ± 0.18b                                   | $2.75 \pm 0.4a$  |
|              |                            | T4                                 | 1.50 ± 0.16ab                            | $1.43 \pm 0.2a$                          | 2.92 ± 0.21a                                   | $1.02 \pm 0.1b$  |
|              | 20                         | СК                                 | $2.01 \pm 0.08b$                         | 0.23 ± 0.03d                             | 2.22 ± 0.11c                                   | 8.40 ± 1.6a      |
|              |                            | T1                                 | 2.15 ± 0.32b                             | 0.62 ± 0.13b                             | 2.79 ± 0.15b                                   | 2.94 ± 1.70b     |
|              |                            | T2                                 | 2.43 ± 0.2ab                             | 0.51 ± 0.06bc                            | 2.96 ± 0.13ab                                  | 3.87 ± 1.31b     |
|              |                            | T3                                 | 2.82 ± 0.3a                              | 0.31 ± 0.06 cd                           | 3.28 ± 0.20a                                   | 7.96 ± 3.20a     |
|              |                            | T4                                 | 2.19 ± 0.3b                              | $0.92 \pm 0.1a$                          | 3.11 ± 0.17a                                   | $1.52 \pm 0.7b$  |
| Zhangzagu 10 | 10                         | СК                                 | 1.50 ± 0.02c                             | 0.25 ± 0.08c                             | 1.77 ± 0.13d                                   | 5.33 ± 2.04a     |
|              |                            | T1                                 | 1.78 ± 0.11c                             | 0.73 ± 0.1ab                             | 2.50 ± 0.25c                                   | $2.44 \pm 0.22b$ |
|              |                            | T2                                 | 2.43 ± 0.12b                             | 0.71 ± 0.3ab                             | 3.09 ± 0.16b                                   | 2.75 ± 1.85b     |
|              |                            | T3                                 | 2.47 ± 0.11b                             | $1.00 \pm 0.2a$                          | 3.55 ± 0.09a                                   | 2.57 ± 0.70b     |
|              |                            | T4                                 | $2.74 \pm 0.04a$                         | 0.61 ± 0.0b                              | 3.40 ± 0.13ab                                  | 3.51 ± 0.56ab    |
|              | 20                         | СК                                 | $1.40 \pm 0.20c$                         | 0.48 ± 0.0.1b                            | 1.88 ± 0.13d                                   | $3.05 \pm 0.7a$  |
|              |                            | T1                                 | 2.16 ± 0.08b                             | 0.48 ± 0.04b                             | 2.60 ± 0.12c                                   | 3.30 ± 0.19a     |
|              |                            | T2                                 | 2.26 ± 0.07ab                            | 0.69 ± 0.16b                             | 2.78 ± 0.09b                                   | 3.44 ± 11a       |
|              |                            | T3                                 | 2.36 ± 0.26ab                            | 0.87 ± 0.40ab                            | 3.28 ± 0.14b                                   | 2.67 ± 2.11b     |
|              |                            | T4                                 | 2.60 ± 0.13a                             | 1.21 ± 0.31a                             | 3.76 ± 0.21a                                   | $2.35 \pm 0.68b$ |

Different letters indicate significant differences at P < 0.05 in the same column and variety. The same as below.

## Table 5

Effects of atrazine on GSH, GSSG, total GSH and GSH/GSSG ratio of foxtail millet.

| Varieties    | Application of days (d) | Treatments (g·hm <sup>-2</sup> ) | GSH content<br>(µmol∙g <sup>−1</sup> FW) | GSSG content<br>(µmol·g <sup>-1</sup> FW) | Total GSH content<br>(µmol·g-1 FW) | GSH/GSSG        |
|--------------|-------------------------|----------------------------------|--|---|------------------------------------|-----------------|
| Jingu 21     | 10                      | СК                               | 4.40 ± 0.01c                             | 0.59 ± 0.2ab                              | 6.0 ± 0.03b                        | 8.01 ± 0.1b     |
|              |                         | T1                               | 4.41 ± 0.02c                             | 0.61 ± 0.0a                               | 6.01 ± 0.03b                       | 7.85 ± 0.2b     |
|              |                         | T2                               | $4.46 \pm 0.04b$                         | 0.60 ± 0.05a                              | 6.09 ± 0.04a                       | 8.07 ± 0.3b     |
|              |                         | T3                               | $5.46 \pm 0.05b$                         | 0.61 ± 0.06a                              | 7.05 ± 0.01a                       | 7.85 ± 0.5b     |
|              |                         | T4                               | 5.52 ± 0.02a                             | $0.55 \pm 0.04b$                          | 7.03 ± 0.01a                       | 9.08 ± 0.4a     |
|              | 20                      | СК                               | 5.52 ± 0.02c                             | 0.58 ± 0.03ab                             | 5.10 ± 0.03d                       | 8.33 ± 0.06ab   |
|              |                         | T1                               | 5.56 ± 0.02b                             | 0.56 ± 0.02b                              | 6.12 ± 0.1 cd                      | 8.78 ± 0.8a     |
|              |                         | T2                               | 5.56 ± 0.01b                             | 0.66 ± 0.32a                              | 6.20 ± 0.3ab                       | 7.95 ± 0.4b     |
|              |                         | T3                               | 5.69 ± 0.02a                             | 0.61 ± 0.01ab                             | 6.40 ± 0.1a                        | 8.23 ± 0.2ab    |
|              |                         | T4                               | 5.52 ± 0.01b                             | 0.59 ± 0.02b                              | 6.18 ± 0.02bc                      | 8.51 ± 0.06a    |
| Zhangzagu 10 | 10                      | CK                               | 1.50 ± 0.01d                             | 0.26 ± 0.07c                              | 1.74 ± 0.10d                       | 5.30 ± 2.05a    |
|              |                         | T1                               | 1.77 ± 0.12c                             | 0.74 ± 0.11ab                             | 2.46 ± 0.23c                       | 2.41 ± 0.1b     |
|              |                         | T2                               | 2.39 ± 0.12b                             | 0.74 ± 0.29ab                             | 3.17 ± 0.15b                       | 2.78 ± 1.84b    |
|              |                         | T3                               | 2.44 ± 0.1b                              | 1.00 ± 0.26a                              | 3.50 ± 0.07a                       | 1.50 ± 0.5c     |
|              |                         | T4                               | 2.72 ± 0.03a                             | $0.66 \pm 0.08b$                          | 3.35 ± 0.10ab                      | 3.5 ± 0.5ab     |
|              | 20                      | CK                               | 1.40 ± 0.21c                             | 0.49 ± 0.06b                              | 1.91 ± 0.16d                       | $4.08 \pm 0.5a$ |
|              |                         | T1                               | $2.10 \pm 0.09b$                         | 0.48 ± 0.06b                              | 2.64 ± 0.12c                       | 3.60 ± 0.1a     |
|              |                         | T2                               | 2.37 ± 0.07ab                            | 0.67 ± 0.18b                              | 2.92 ± 0.10b                       | 2.40 ± 1.01b    |
|              |                         | T3                               | 2.35 ± 0.16ab                            | 0.85 ± 0.41ab                             | 3.20 ± 0.19b                       | 3.41 ± 2.3a     |
|              |                         | T4                               | 2.62 ± 0.11a                             | 1.28 ± 0.30a                              | 3.80 ± 0.21a                       | $2.28 \pm 0.7b$ |

Different letters indicate significant differences at P < 0.05 in the same column and variety. The same as below.

# Table 6

Effects of atrazine on yield and yield components of foxtail millet.

| Varieties | Treatments (g•hm <sup>-2</sup> ) | Ear length<br>(cm) | Ear diameter<br>(mm) | Spike weight<br>(g) | Grain weight<br>(g) | Ear yardage     | 1000-grains<br>Weight<br>(g) | Number of<br>ears<br>(10 <sup>4</sup> ·hm <sup>-2</sup> ) | Yield<br>(kg∙667 m²) |
|-----------|----------------------------------|--------------------|----------------------|---------------------|---------------------|-----------------|------------------------------|---|----------------------|
| Jingu 21  | СК                               | 24.61 ± 2.25a      | 33.79 ± 2.87a        | 34.50 ± 1.5a        | 26. 88 ± 1.13a      | 109.87 ± 7.8a   | 3.11 ± 0.3a                  | 27.65 ± 1.6a  | 540.6 ± 15.5a        |
|           | T1                               | 23.47 ± 2.33a      | 31.96 ± 2.95a        | 34.32 ± 2.5a        | 22.74 ± 1.34a       | 108.31 ± 6.2a   | 3.06 ± 0.212a                | 26.30 ± 1.11a   | 458.1 ± 13.1b        |
|           | T2                               | 23.79 ± 2.12a      | 30.80 ± 3.64a        | 32.19 ± 1.8ab       | 23.90 ± 1.91b       | 104.09 ± 4.9ab  | 2.07 ± 0.21a                 | 26.00 ± 2.0a  | 459.6 ± 15.7c        |
|           | T3                               | 23.42 ± 2.06a      | 28.65 ± 3.30ab       | 31.75 ± 1.8b        | 22.19 ± 1.80c       | 100.21 ± 6.5ab  | 2.01 ± 0.12a                 | 20.66 ± 2.4b  | 358.88 ± 6.8d        |
|           | T4                               | 23.13 ± 1.50a      | 26.88 ± 4.42b        | 28.70 ± 3.5c        | 23.95 ± 1.30b       | 99.64 ± 8.9b    | 2.02 ± 0.01a                 | 16.66 ± 1.51bc  | 271.0 ± 9.83e        |
| Zhangzagu | CK                               | 31.28 ± 1.60ab     | 33.30 ± 1.3a         | 31.17 ± 2.1a        | 23.92 ± 2.14a       | 117.44 ± 7.7a   | 4.25 ± 0.21a                 | 32.00 ± 1.00a   | 577.0 ± 8.09a        |
| 10        | T1                               | 32.53 ± 2.92a      | 32.06 ± 3.1a         | 28.79 ± 3.1b        | 21.86 ± 2.33ab      | 114.88 ± 3.8ab  | 4.122 ± 0.13ab               | 29.30 ± 2.8ab   | 568.0 ± 9.60a        |
|           | T2                               | 28.08 ± 3.20ab     | 30.32 ± 2.5ab        | 26.68 ± 2.3bc       | 20.70 ± 3.01bc      | 113.57 ± 5.2ab  | 3.10 ± 0.32ab                | 30.33 ± 5.0abc  | 411.0 ± 8.15b        |
|           | T3                               | 27.9 ± 3.69ab      | 30.02 ± 3.2b         | 25.62 ± 1.1c        | 17.81 ± 2.5 cd      | 110.87 ± 5.0b   | 2.05 ± 0.12ab                | 26.69 ± 2.72bc  | 340.40 ± 9.9c        |
|           | T4                               | 26.02 ± 3.20b      | 28.56 ± 2.5b         | 22.20 ± 1.1d        | 16.29 ± 1.80d       | 102.30 ± 8.06bc | 2.95 ± 0.21b                 | 22.65 ± 2.6c  | 275.21 ± 10.3d       |

Different letters indicate significant differences at P < 0.05 in the same column and variety. The same as below.

effect due high doses. The growth inhibition of atrazine is similar to the accumulation of  $O_2^-$  and  $H_2O_2$  in plants (Guo et al., 2010). Our results are supported by (Guo et al., 2019; Sun et al., 2019) that as post-emergence herbicide like fluroxypyr and Bensulfuronmethyl (BSM) reduced growth parameters so as atrazine concentrations increased the growth parameters also decreased in our study (Ning et al., 2015). Chlorophyll pigments in plants absorb light energy that is used for producing photosynthates. For example, chlorophyll content is the main source of light energy for photosynthesis. Increased doses of atrazine in foxtaill millet significantly reduced the photosynthetic content of the leaves (Su et al., 2016; Guo et al., 2005). Increased atrazine doses reduced growth parameters such as plant height and biomass, which reduced stomatal conductance, respiration, and reduced photosynthesis. Our findings proved that as atrazine doses increased there was significant decrease were recorded in Fv/Fm. FPSII. Fo. Fv/ Fm, qP, and ETR in foxtail millet (Wu and Bao, 2011; Guo et al., 2019). PSII and ETR reduction at atrzine treatment increase was due to a reduction of the efficiency of excitation energy capture of PSII reaction centers (Wu and Bao, 2011). The reduction of qP as atrazine doses increase was due to that atrazine harmed PSII reaction centers and increased the proportion of closed PSII reaction centers, probably cause a decrease in the proportion of available excitation energy used for photochemistry (Bigot et al., 2007). Our findings are supported by Yuan et al., 2013; 2017, who reported that sigma broad in Radix Isatidis seedlings and foxtail millet.

Atrazine is photosynthetic inhibitor as it stop the electronacceptor protein PSII by reducing electron transfer in the photosynthetic process. This reduction of electron stop the chlorophyll-absorbed light energy into electro-chemical energy, which is main cause of reduction of chlorophylls and oxygens (Perez-Jones et al., 2009; Erinle et al., 2016). The high production of these ROS reduces synthesis of proteins, lipids and pigments (Erinle et al., 2016). The main reason of this is that herbicide application to plants may be destroy antioxidant defense system. Same results are reported in wheat that overproduction of ROS is due to herbicide application (Jiang and Yang 2009) and in rice seedlings by over accumulation of atrazine in rice shoot (Zhang et al., (2014).

Atrazine effect on foxtail millet leaves for enzymes produces free radicals, that will damage the cells. ROS (free redicals) are damaged by activation of antioxidant enzymes. One enzyme SOD catalyzes the molecules of superoxide into  $H_2O_2$  and  $O_2$ . In this study, effect of atrazine (50 mg/kg) on SOD performance in foxtail millet seedlings enhanced and then reduced, the main reason of this is damage of defense system in the leaves. Moreover, POD enzyme is also used in the split of  $H_2O_2$  (Wang and Zhou 2006). This will increase POD activity in the foxtail millet to atrazine and POD and CAT are enzymes breakdown  $H_2O_2$  into water (Yin et al. 2008) in plants. CAT/POD enzymes also work together to remove  $H_2O_2$  at high level with less power use (Siddiqui et al., 2011; Xu et al., 2013). The CAT/POD enzymes protect the photosynthesis against oxidative stress (Liang et al., 2009).

Moreover CAT enzymes reduced in foxtail millet as atrazine concentration increased. This is reduction in CAT activity is by increasing  $H_2O_2$  that reduces the enzyme activity (Song et al., 2007). Activation of POD and CAT enymes increases defense system of plant to protect the oxidative stress caused by atrazine (Mi et al., 2014; Qiu et al., 2008; Liu et al., 2011).

# 5. Conclusion

According to our results, as atrazine application increased growth parameters like plant height and biomass decreased which reduced chlorophyll fluorescence parameters. The overall photosynthetic performance index, PI, and Fv/Fm in our study were considered very sensitive indicator in response to photosynthetic performance of foxtail millet varieties. Our present findings showed that increased atrzine concentrations reduced the photosynthetic performance in foxtail millet varieties. Fianlly, according to our results high doses of atrazine badly effected physiological process of foxtail millet. High doses of atrazine promoted the photosynthesis process by whic activities of the antioxidant enzymes were reduced, in order to protect ROS production. It is very important that find suitable atrazine concentrations which is safe for physiological damages to the plant. We recommended that T2 and T3 treatments of atrazine are safe for foxtaill millet cultivation and among cultivars zhangzagu 10 performed better.

# **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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