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The Effect of Irrigation Patterns on Flavonoid and Phenol Content in Three Sorghum Varieties

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A B S T R A C T

Global climate change, characterized by increasing drought intensity, poses a serious threat to the sustainability of agricultural production. Sorghum (Sorghum bicolor L.) is a strategic drought-tolerant commodity, but studies on its secondary metabolite response are still limited, especially in superior varieties. This study aims to analyze the effect of drought stress on the flavonoid and phenol content in water extracts of Super 1, Mandau, and Suri 4 sorghum varieties. The three varieties were planted on coastal land with four different irrigation frequencies (daily, every 4 days, every 5 days, and every 6 days), then extracted and analyzed for flavonoid and phenol content using spectrophotometry. The results of the ANOVA analysis showed that drought stress had a very significant effect on flavonoid and phenol content ($p < 0.05$). The Super 1 variety with irrigation every 5 days produced the highest flavonoid content, while the highest phenol accumulation was obtained in the combination of Super 1 and irrigation every 6 days. Conversely, conditions without stress (daily irrigation) reduced the content of both metabolites, especially in the Mandau variety. These findings confirm that moderate to severe water stress can increase the biosynthesis of secondary metabolites as an adaptive defense mechanism in plants

INTRODUCTION

Global climate change, characterized by rising temperatures and declining water availability, has led to increasingly severe droughts, posing a serious challenge to the agricultural sector, especially for food crops that are highly dependent on water availability (Chiappini et al., 2025; Zhang & Guo, 2025).

Therefore, there is a need for commodities that are adaptive to abiotic stresses such as drought, one of which is sorghum (Khalifa & Eltahir, 2023). As a cereal crop, sorghum tolerates marginal land and dry conditions. It has excellent potential as a source of food, animal feed, and bioenergy raw material (Patil et al., 2021). In Indonesia, superior varieties such as Sorghum Super 1 have been widely developed due to their high productivity and adaptability to various environments. However, the utilization of sorghum to date has still focused on seed production, while studies on its secondary metabolite content are relatively limited.

Drought can trigger oxidative stress in plants, characterized by increased formation of Reactive Oxygen Species (ROS) (Panda et al., 2024). In response, plants activate the biosynthesis pathway of secondary metabolites—including flavonoids and phenolic compounds—which increase during drought and function as an endogenous antioxidant system to neutralize ROS and protect cellular components from oxidative damage (Yin et al., 2025). These findings align with a study by Susilo et al. (2024), which reported that drought stress in the Mandau variety of sorghum significantly increased flavonoid and phenolic content as an adaptive defense mechanism against ROS accumulation. In addition to their role in defense mechanisms, flavonoids and phenolics also have potential commercial and ecological applications as raw materials for pharmaceuticals, functional foods, and sources of natural bioherbicides/allelopathic compounds (Kostina-Bednarz et al., 2023).

To date, most research on sorghum has focused on its morphological and physiological responses to drought, such as changes in plant height, water use efficiency, and photosynthetic activity (Kamali et al., 2023; Mbuma et al., 2024). Conversely, in-depth studies on the dynamics of secondary metabolite content, such as flavonoids and phenolic compounds, under drought stress conditions, particularly in the superior Sorghum Super 1 variety, are still minimal and have not been comprehensively explored. In fact, this information is crucial for understanding the biochemical adaptation mechanisms of plants to oxidative stress and has the potential to open up opportunities for utilizing sorghum as a source of value-added bioactive compounds, including antioxidants and natural bioherbicides (Yue et al., 2025; Awika, 2022). Susilo et al. (2024) also emphasize that the secondary metabolite response in sorghum is greatly influenced by the intensity and duration of stress, and varies between cultivars.

Based on this, this study aims to examine the effect of drought stress on the flavonoid and phenolic content in Super 1 sorghum extracts (Yue et al., 2025). This study is important considering that increased biosynthesis of secondary metabolites such as flavonoids and phenolics has been reported as one of the biochemical defense mechanisms of plants against oxidative stress due to drought (Mbuma et al., 2024; Yin et al., 2025; Susilo et al., 2024). The results obtained are expected to expand the use of sorghum as a food commodity and as a source of economically valuable bioactive compounds to support the development of sustainable agriculture and bio-based industries (Cunha et al., 2024; Awika, 2022).

LITERATURE REVIEW

Sorghum is a cereal crop that can adapt to dry climates and grow well in marginal lands. During drought, this plant increases its production of secondary metabolites such as phenolics, flavonoids, and terpenoids. These compounds are natural defense mechanisms against pathogens, herbivores, and other pests (Akula & Ravishankar, 2011). One of the phenolic metabolites produced by sorghum roots is sorgoleone, an allelopathic compound that inhibits the germination and growth of surrounding weeds. The production of sorgoleone and other secondary metabolites increases significantly due to drought stress, strengthening sorghum's competitive ability against other plants. Drought conditions trigger oxidative stress and activate enzymes in the phenylpropanoid biosynthesis pathway, ultimately increasing the accumulation of phenolic compounds, including sorgoleone. Thus, sorghum has greater potential as a natural bioherbicide (Khan et al., 2020).

Sorghum is naturally effective at suppressing weeds, especially in dry environments prone to drought, making it very useful as a natural weed control agent (Weston & Czarnota, 2001). This ability can reduce dependence on chemical herbicides that have the potential to pollute the environment. Therefore, it is important to note that drought stress on sorghum plays a significant role in increasing the production of allelopathic secondary metabolites that have the potential to be used as natural bioherbicides. Further research is needed to identify the specific active compounds responsible for the allelopathic effect and to formulate strategies for its application in broader, sustainable, and environmentally friendly agricultural systems (Susilo et al., 2023; Susilo et al., 2024; Susilo et al., 2021).

Akula and Ravishankar (2011) stated that abiotic stress, such as drought, has increased the production of secondary metabolites in plants, including flavonoids. Flavonoids function as natural antioxidants that protect plants from oxidative stress. Khan et al. (2020) reported that drought activates key enzymes in the phenylpropanoid biosynthesis pathway that produce flavonoids. That flavonoids play an important role in protecting plant cells from damage caused by drought, with their production increasing in response to environmental changes. Phenolic acids, one of the derivatives of phenolic compounds, have been widely recognized as allelopathic agents and have great potential to be developed as environmentally friendly bioherbicides (Hoang Anh et al., 2021).

METHODOLOGY

Mandau, Super 1, and Suri 4 sorghum varieties were cultivated on coastal land with different watering frequencies, namely every day, every four, every five, and every six days. At 40 days of age, the plants were harvested by separating the tops from the roots, then dried in the sun for 14 days. Next, the material was dried in an oven at 70 °C for 72 hours. The dry samples were cut into 1–2 cm pieces, ground into a fine powder, and used as material for making water extracts to analyze flavonoid and phenol content.

The flavonoid content is determined by dissolving 10 mg of extract powder in 10 ml of solvent. A total of 0.5 ml of the solution is mixed with 1.5 ml of 96% ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M CH_3COONa, and 2.8 ml of distilled water. The mixture was incubated for 30 minutes, then the absorbance was measured at a wavelength of 435 nm using a standard series treated with an identical method.

Phenol content analysis was performed by dissolving 10 mg of extract in a 10 ml volumetric flask. A total of 0.2 ml of solution was taken, mixed with 15.8 ml of distilled water, then 1 ml of Folin–Ciocalteu reagent was added and incubated for 8 minutes. After that, 3 ml of 20% Na_2CO_3 was added and left for 30 minutes before the absorbance was measured at a wavelength of 765 nm, using a standard curve with the same treatment.

The study used a completely randomized design (CRD) with one factor and four replicates. The main variables observed were flavonoid and phenol content. The data were analyzed using ANOVA and followed by a BNT test at a 5% confidence level ($P < 0.05$) if significant differences were found.

RESULTS AND DISCUSSION

The variables observed in this study included flavonoid and phenol levels in three extracts of Mandau, Super 1, and Suri 4 varieties that had undergone different levels of drought stress. The analysis of variance (Table 1) shows that the treatment of sorghum water extracts with different levels of drought stress had a significant effect on flavonoid and phenol levels.

Table 1. Recapitulation of Analysis of Variance of Flavonoid and Phenol Levels

Observation variables	Treatment	Coefficient of variation (CV)
Flavonoid	4.80 **	33.61
Fenol	4.61 **	29.86
** = berpengaruh sangat nyata Tn = tidak berpengaruh nyata		

Treatment with extracts from three sorghum varieties significantly affected flavonoid content. The highest flavonoid content was achieved by extracts from Super 1 with a watering pattern every 5 days. However, this was not significantly different from extracts from Mandau with a watering pattern every 6 days and was significantly different from other treatments. The extract from Super 1 with a watering pattern every 6 days achieved the lowest flavonoid content. However, it was not significantly different from Mandau with a daily watering pattern, Mandau with a watering pattern every 5 days, Super 1 with a daily irrigation pattern, Suri 4 with a daily irrigation pattern, Suri 4 with a 5-day irrigation pattern, and Suri 4 with a 6-day irrigation pattern, as shown in Table 2.

The results showed that the treatment of extracts from three sorghum varieties significantly affected flavonoid content (Xu & Zheng, 2021). The highest flavonoid values were obtained from extracts derived from the Super 1 variety with a watering pattern every 5 days, indicating that mild water stress can increase the accumulation of secondary metabolites (Pinheiro et al., 2021). Interestingly, the flavonoid content was not significantly different from the extract from the Mandau variety irrigated every 6 days, indicating a similar adaptive physiological response between the two varieties to water stress (Cunha et al., 2024).

Conversely, the lowest flavonoid levels were found in Super 1 extracts with a watering pattern every 6 days. However, this value was not significantly different from Mandau (daily and every 5 days watering), Super 1 (daily watering), or all treatments in the Suri 4 variety (daily, 5 days, and 6 days watering), as shown in Table 2. These findings reinforce that the interaction between genotype and water availability greatly determines flavonoid accumulation, where moderate stress increases phenolic metabolite production, while excessive stress decreases it (Wu et al., 2017; Ni et al., 2025).

Table 2. Average Flavonoid and Phenol Content in Extracts of three Sorghum Varieties with Different Levels of Drought Stress

Irrigation treatment	Flavonoid (µg/g)	Fenol (µg/g)
Mandau + daily irrigation	415.0 c	1085.0 d
Mandau + irrigation every 5 days	395.0 c	1560.0 cd
Mandau + irrigation every 6 days	770.0 ab	2455.0 abc
Super 1 + daily irrigation	330.0 c	1150.0 d
Super 1 + irrigation every 5 days	1045.0 a	2050.0 bcd
Super 1 + irrigation every 6 days	320.0 c	3310.0 a
Suri 4 + daily irrigation	520.0 bc	2790.0 ab
Suri 4 + irrigation every 5 days	615.0 bc	2970.0 ab
Suri 4 + irrigation every 6 days	570.0 bc	1950.0 bcd

Note: Numbers followed by the same letter in the same column are not significantly different in the 5% LSD test

Treatment with extracts from three sorghum varieties significantly affected phenol content. The extract from Super 1 with a watering pattern every 6 days achieved the highest phenolic content. However, it was not significantly different from the extract from Mandau, which had a watering pattern every 6 days, and Suri 4 every 4 days. Suri 4 with a watering pattern every 5 days was significantly different from the other treatments. The extract from Mandau with a daily irrigation pattern achieved the lowest phenolic content. However, it was not significantly different from Mandau with a 5-day irrigation pattern, Super 1 with a daily irrigation pattern, Super 1 with a 5-day irrigation pattern, and Suri 4 with a 6-day irrigation pattern, as shown in Table 2.

The analysis results show that the treatment of extracts from three sorghum varieties significantly affected total phenolic content (Xu & Zheng, 2021). The highest phenolic concentration was obtained in the Super 1 extract with a watering pattern every 6 days, indicating that moderate to severe water stress can induce an increase in the biosynthesis of phenolic compounds as a plant defense mechanism (Cunha et al., 2024; Pinheiro et al., 2021). However, this value was not significantly different from the Mandau treatment (irrigation every 6 days) or Suri 4 with irrigation every 4 days and 5 days, indicating that the response to increased phenols is not only determined by genotype but also by the appropriate or optimal level of stress (Ni et al., 2025).

Conversely, the lowest phenolic content was found in Mandau extracts with daily irrigation, reflecting that abundant water availability tends to reduce secondary metabolite production because plants have comfortable physiological conditions and do not need to activate metabolic defense pathways (Wu et al., 2017). However, these levels were also not significantly different from Mandau (5-day irrigation), Super 1 (daily and 5-day irrigation), and Suri 4 (6-day irrigation), as shown in Table 2. These findings confirm that the genotype × water stress level interaction strongly determines total phenol production. That moderate stress tends to trigger higher phenol accumulation than excess water or drought conditions.

CONCLUSION AND RECOMMENDATION

The Super 1 variety tended to produce the highest flavonoid content with a watering pattern of every 5 days, while the lowest flavonoid content appeared in the combination of Super 1 and a watering pattern of every 6 days. Meanwhile, the highest phenol content was obtained from extracts of the Super 1 variety with a watering pattern every 6 days. In contrast, the lowest phenol content was found in the Mandau variety with a daily watering pattern. These findings indicate that increased drought stress in general can induce the accumulation of secondary metabolites. However, the optimal response differs between varieties and depends on a level of stress that is not too extreme.

FUTHER STUDY

This research still has delays, so it is necessary to conduct further research related to the topic The Effect of Irrigation Patterns on Flavonoid and Phenol Content in Three Sorghum Varieties in order to improve this research and add insight for readers.

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