



## Effects of rice husk charcoal on the resistance of Jali (*Coix lacryma-jobi* L.) to drought stress during generative phase

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### Abstract

The production of jali (*Coix lacryma-jobi* L.) under stress can be increased by the application of a soil enhancer, called rice husk charcoal. This study aimed to determine the effect of rice husk charcoal on the yield of jali plants under drought stress and to determine the minimum soil water content from the interval time of watering to ensure that plants can still produce grains. The treatment (interval time watering and weight of rice husk charcoal) was arranged in a split-plot design. The main plot was the interval time of watering, consisting of 3 levels: every two days, every four days, and every six days. The weight of rice husk charcoal as sub-plots consisted of 3 levels: 0 t ha<sup>-1</sup>, 10 t ha<sup>-1</sup>, and 20 t ha<sup>-1</sup>. The data were analyzed using analysis of variance (ANOVA) and further tested using Duncan Multiple Range Test (DMRT) at a significance level of 5%. The results showed that rice husk charcoal could not increase the yield of jali plant under drought stress. Jali plants could still produce grains at a watering interval time of every six days with a minimum soil moisture content of 11.85%, or equivalent to 35.54% of field capacity.

## INTRODUCTION

Indonesia is the fourth most populous country after China, India, and America. The Ministry of National Development Planning of the Republic of Indonesia (2013) predicted that by 2035, Indonesia's population would reach 305 million people. Therefore, the increase in population must be balanced with food availability. So far, rice is the superior staple food of the Indonesian people. Meeting domestic food necessities also sometimes needs to be done by importing rice (Pratama et al., 2019). The Food Consumption Diversification (FCD) Program is intensified to maintain food availability. FCD program does not completely replace the role of rice but changes food consumption patterns. The program aims to reduce dependence on one type of food commodity while improving the nutritional quality of the community (Mani, 2020). This activity is also one of the strategies

to deal with the Corona Virus Disease 2019 (Covid-19) pandemic. Numerous sources of cereal food other than rice that can support the FCD program are available, one of which is jali (*Coix lacryma-jobi* L.).

Jali comes from East Asia and is spread out in many ecosystems in Indonesia. From generation to generation, jali has been known as a local plant. It is used as an alternative food and functional food in several areas of Indonesia including East Kalimantan, West Java, and Central Java. It also has many health benefits and has long been used in traditional medicine. In terms of nutrition, jali grains contain vitamin B1, fat, and protein higher than other cereals. In addition, the calcium content of jali is also higher than sorghum, rice, and corn (Yulianto et al., 2006; Histifarina et al., 2019). The advantage of jali cultivation is its high adaptability to abiotic stresses, especially drought stress. Several studies have shown this plant's high tolerance to drought stress conditions.

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Mahdya et al. (2020) compared watering intervals of one day with that of every four days in jali plants as an approach to drought stress. The results showed that the decrease in growth and yield of jali grains occurred at a watering interval of every four days. However, the plants could still produce grains under irrigation deficit. Fauzi et al. (2020) also revealed that jali plants grown from grains resulted in better growth and grain production when watered every day compared to those watered every four days. Nevertheless, the minimum soil moisture content the jali plants could endure under drought stress was not discussed.

Drought stress lasting during the vegetative and generative periods decreases plants biological and economic yield (Gomaa et al., 2021). The negative effect of drought stress on yield reduction can be reduced by adding husk charcoal to the growing media. Husk charcoal is an organic material that utilizes agricultural waste. Rice husk charcoal was chosen due to its easy preparation process for farmers. Rice husks were burned slowly with steam until the husks turned black, forming charcoal. In addition, husk charcoal is also more resistant to the decomposition process after being applied to planting media (Ali et al., 2018). Husk charcoal application can be used for several growing seasons.

According to Boy et al. (2020), entisol soil (including regosol) has a soil moisture content of 33.34% of field capacity and a permanent wilting point soil moisture content of 8.14%. According to Hastuti et al. (2017), regosol soil has some characteristics: (1) sand fraction for most of its constituents, (2) low soil surface area, and (3) high porosity, thereby reducing the soil ability to water holding capacity and nutrients retention. Mishra et al. (2017) revealed that the surface area of RHC was higher than that of soil or rice husk. Husk charcoal added in the growing media increases the specific surface area of soil. This increases the ability of the soil to hold water and slow-release nutrient. This study aimed to determine the effect of rice husk charcoal on the yield of jali plants under drought stress and to determine the minimum soil water content from the interval time of watering to ensure that plants still can produce grains.

## MATERIALS AND METHODS

The field research was conducted at Krodan Sub-village, Maguwoharjo Village, Depok, Sleman, Yogyakarta

using a screen house with a UV-plastic roof. Depok District has an altitude of 100 – 499 meters above sea level (masl) and is dominated by regosol soil types. Research steps consist of planting, fertilization, watering, field observation, sampling, and harvesting, until laboratory observation. The planting was carried out in January 2021, and the generative phase of the jali plant started in April–June 2021. Field observations were carried out seven days before the plant entered the generative phase, which was 88 days after planting (DAP) until the first grain was harvested at 148 DAP. Observation of destructive sample plants has been done at the Laboratory of Plant Production Management, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

The field equipment and materials in this study were ma-yuen variety jali seeds, rice husk charcoal, regosol soil, inorganic fertilizers (Urea 2.58 g/polybag, KCl 0.49 g/polybag, TSP 0.82 g/ polybag), and irrigation water for irrigation treatment. The tools used in this study were polybags sized 40 cm x 40 cm, a hoe, shovel, scissors, label, plastic, stationery, and meter, while the laboratory tools used consisted of an analytical scale, mortar, Whatman paper, reaction tube, microtube, microtip, micropipette, vortex, spectrophotometer, and oven. The experiment was arranged using a split-plot design. The interval time of watering as the main plot consisted of three levels, namely every two days, every four days, and every six days. The weight of husk charcoal as sub-plots comprised three levels, namely 0 t ha<sup>-1</sup>, 10 t ha<sup>-1</sup>, and 20 t ha<sup>-1</sup>. Each treatment combination had three replications. The observational data were analyzed for variance (ANOVA) and then, significant ANOVA results were tested using Duncan Multiple Range Test (DMRT) ( $\alpha = 0.05$ ). Data analysis was done using R Studio software, while data visualization was performed using Microsoft Excel software.

### Soil moisture content (%)

The treatment of interval time of watering started since 28 DAP up to 7 days before harvest. A sampling of soil moisture content was carried out at 100, 112, 136, and 148 DAP. Soil moisture content was observed in two stages (before and after watering). Soil moisture content (SMC) was determined based on the gravimetric method by taking a sample of  $\pm 10$  g of soil in the treatment pot, as a result of fresh weight (FW). The soil sample was then heated in an oven until the absolute dry weight (DW) was reached. Soil

moisture content was calculated using equation 1.

$$\text{SMC} = [(FW-DW)] \times 100 \dots\dots\dots (1)$$

**Relative water content (%)**

Relative Water Content (%) of leaves was measured by weighing 10 grams of leaves from each sample plant to determine the fresh weight (FW). The leaves were immersed in water in a plastic bag tied to full turgidity for 24 hours and reweighed to determine the leaf-saturated weight (SW). The leaves were then dried in an oven at 80°C for 48 hours until the weight was constant to determine the leaf dry weight (DW). RWC was calculated by equation 2.

$$\text{RWC} = [(FW-DW)-(SW-DW)] \times 100 \dots\dots\dots (2)$$

**Transpiration period (minutes)**

Transpiration period (minutes) was measured using the cobalt chloride paper method. Before being used, Whatman No 1 filter paper was cut into 3 cm x 3 cm in size. The paper was then given a solution of cobalt chloride at a concentration of 0.05% (1 g in 20 ml of water). The water content of the paper pieces was removed using an oven until they turned pink. The paper was then placed in a closed container and ready to be used for testing plant transpiration rates. The cobalt chloride paper was on the underside of the leaf and clamped together with transparent plastic so the paper clip did not damage the leaf layer. The transpiration period was expressed in minutes. Afterwards, the time was recorded when the colour changed from blue to pink evenly. The longer it took to change the colour of the paper, the less water was evaporated from the surface of the plant's leaves. Temperature, light intensity, and humidity at the time of sampling were also observed.

**Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was determined by weighing 0.5 grams of fresh leaves that had been crushed with liquid nitrogen. Then 0.1% trichloroacetic acid (TCA) leaf extract supernatant, 100 mM K-phosphate buffer, and reagent 1 M KI w/v in H<sub>2</sub>O were added with a 1:1:4 ratio from the leaves weight. The solution mixture was left for 1 hour in the dark. The absorbance was measured at a wavelength of 390 nm with 0.1% of TCA without leaf extract as a blank. The measurement of the amount of H<sub>2</sub>O<sub>2</sub> was calculated using the standard curve equation made with several concentrations of H<sub>2</sub>O<sub>2</sub> (Alexieva et al., 2001).

**Proline (µmol proline g<sup>-1</sup>)**

Proline (µmol proline g<sup>-1</sup>) levels were measured based on the ninhydrin test method. The ninhydrin test was conducted by taking and crushing fresh leaves sample of about 0.5 g using a mortar. Then, 10 ml of sulfosalicylic solution (3%) was poured into the mortar and stirred until evenly distributed. The filtrate was filtered into a new container using filter paper (Whatman paper). 2 ml of the filtrate was taken to add reagents, namely ninhydrin acid and glacial acetic acid in a ratio of 1:1:1 with the volume of the filtrate and then incubated for 1 hour at a temperature of 100°C. After 1 hour, the test tube was placed into cold water to stop the reaction. The reaction sample was then extracted by adding 4 ml of toluene and shaken using a vortex until two separate liquid layers were formed (15–20 seconds). The red toluene containing proline was separated with a pipette, and its absorbance was measured using a spectrophotometer at a wavelength of 520 nm (Bates et al., 1973).

**Superoxide dismutase (unit ml<sup>-1</sup>)**

Superoxide dismutase (unit ml<sup>-1</sup>) was determined based on the pyrogallol autoxidation method by Marklund and Marklund (1974). In short, the reaction medium was a mixture of 50 mM Tris-HCl buffer (pH 8.2), and 1 mM EDTA was added with protein extract from the test sample (40-60 mg). To start the reaction, 100 µL of pyrogallol (0.2 mM) was added to the reaction medium mixture and measured with a spectrophotometer at 420 nm. Then the results of the decrease in pyrogallol absorbance were recorded to determine the SOD activity.

**Harvest Index (HI)**

Harvest Index (HI) is a growth analysis approach to determine the ability of plants to distribute the assimilates to the sink. HI was calculated using equation 3.

$$\text{HI} = [\text{Economic dry weight}/\text{plant dry weight}] \dots (3)$$

**Yield contributing characters**

Yield contributing characters included the number of filled grains was determined by calculating the number of filled grains per plant. The weight of 100 grains per clump (g) was determined by weighing the weight of 100 grains per plant. Total grain weight (g) was determined by weighing the entire weight of grains harvested per plant.

## RESULTS AND DISCUSSION

The treatment of rice husk charcoal and watering time interval on all observed variables has no interaction effect. According to Table 1, the treatment of rice husk charcoal (RHC) independently had no significant effect on the soil moisture content before watering, the transpiration period, and the relative water content of the leaves. However, with or without adding RHC, the soil moisture content before watering decreased by almost 50% of field capacity. This result was presumably because the organic matter just from RHC were not able to improve the characteristics of the regosol soil. Regosol soil is dominated by a sand fraction, so it needs to be combined with other organic matter in the form of manure or compost. The fertilizer serves to help improve the function of the RHC in restoring the soil. In the research conducted by Mustikawati et al. (2019) the low productivity and fertility of the regosol soil could be improved by the addition of organic matter in the form of cow manure. Cow manure was proven to increase the growth of patchouli plants planted in regosol soil. Caroline et al. (2021) also revealed that the combination of giving coffee husk charcoal and cow manure improved the microbiological and chemical properties of regosol soil. The combination of coffee husk charcoal amendments and cow manure increased C:N ratio, the pH content, total organic carbon, potassium, calcium, and phosphor. Seyedsadr et al. (2022) in their research revealed that bio-charcoal also had an impact on nutrient retention in the soil when added together with

manure or compost amendments. Bio-charcoal is a positive effect on low soil fertility affected by drought.

Independently, the interval time of watering treatment significantly affected soil moisture content. Before re-watering, there was a decrease in soil moisture content near the permanent wilting point as the watering time interval increased. The lowest soil moisture content at interval time of watering of every six days reached 11.85%, or equivalent to 35.54% of field capacity (Table 1). The decrease in available water content before watering resulted in a longer transpiration time and a lower relative water content of plants. The response of jali plants to drought stress conditions was to close the stomata. Closure of stomata reduced the rate of water loss through transpiration because stomata are the pathways for transpiration and gas exchange. The decrease in the transpiration rate was associated with the decreasing leaf relative water content. Relative water content is one of indicators that shows the potential status of plant water. Water potential plays an essential role in transporting water from the soil to the plants. Water can be absorbed by plants when the groundwater potential is higher than the plant potential. When there is a drought, the groundwater potential is lower than that of the plant water, so it is difficult for plants to absorb water. Therefore, low water availability and low relative water content on the plant watered every six days indicated that the plant was exposed to drought stress.

The application of RHC increased proline production in plant leaves. The increase in proline production was in line with the decrease in the level of cell

**Table 1.** Soil moisture content (SMC) before and after watering, transpiration, and relative water content (RWC) of *jali* leaf under the different weights of rice husk charcoal and interval time of watering

Treatments	Available water before watering (%)	Percentage field capacity (%)	Decrease of soil moisture (%)	Transpiration (minute)	RWC (%)
<b>Rice husk charcoal</b>					
0 t ha <sup>-1</sup>	18.44 a	55.31 a	43.47 a	7.61 a	68.57 a
10 t ha <sup>-1</sup>	17.52 a	53.13 a	47.25 a	7.28 a	63.78 a
20 t ha <sup>-1</sup>	17.71 a	52.55 a	48.17 a	8.32 a	63.09 a
<b>Interval time of watering</b>					
Every two days	24.36 p	73.06 p	24.46 r	6.16 r	73.36 p
Every four days	17.46 q	52.38 q	48.08 q	7.52 q	63.48 pq
Every six days	11.85 r	35.54 r	66.39 p	9.53 p	58.60 q
Interaction	(-)	(-)	(-)	(-)	(-)
CV (%)	8.74	8.74	10.47	13.85	14.88

Remarks: Based on DMRT ( $\alpha = 0.05$ ), there was no significant difference between the values with the same alphabet in one column, and (-) interaction effects were insignificant.

damage indicated by the level of H<sub>2</sub>O<sub>2</sub> and Malondialdehyde (MDA). The levels of H<sub>2</sub>O<sub>2</sub> and MDA in plants given 10 and 20 t ha<sup>-1</sup> of RHC without RHC were statistically the same. The increase in proline indicates that the plant provides a tolerance mechanism response to the decrease in soil moisture before watering. The increase of proline level in the RHC treatment supported that the provision of RHC organic matter was not good enough to improve the characteristics of the regosol soil. The same thing also happened at intervals of watering treatment.

The longer the interval time of watering, the more increasing the production of proline, while the levels of H<sub>2</sub>O<sub>2</sub> at intervals of watering every 2, 4, and 6 days still produced the same levels statistically. It means that at intervals of watering every 4 and 6

days, proline production could suppress the presence of H<sub>2</sub>O<sub>2</sub>, equivalent to watering every 2 days. The same thing on the decrease in the amount of cell damage which was indicated by the level of malondialdehyde (MDA) was not significantly different between watering intervals of 2, 4, and 6 days (Table 2).

There are three categories of plant adaptation to water deficit: that escape, avoidance, tolerance, or a combination thereof (Sallam et al., 2019). According to Osmolovskaya et al. (2018), an escape mechanism is to complete a plant's life cycle before severe drought stress occurs. The avoidance of plants from drought stress is through reducing the negative impact by increasing the abscisic acid content in the roots. ABA accumulation initiates leaf stomata closure, so the rate of transpiration can be slowed down.

**Table 2.** Content of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide dismutase (SOD), and proline of jali leaves under the different weights of rice husk charcoal and interval time of watering.

Treatments	H <sub>2</sub> O <sub>2</sub> (ppm)	SOD (unit ml <sup>-1</sup> )	Proline (umol proline g <sup>-1</sup> )
<b>Rice husk charcoal</b>			
0 t ha <sup>-1</sup>	21.86 a	0.656 a	0.39 b
10 t ha <sup>-1</sup>	21.27 a	1.277 a	0.61 ab
20 t ha <sup>-1</sup>	20.35 a	0.862 a	1.44 a
<b>Interval time of watering</b>			
Every two days	18.69 p	0.982 p	0.10 q
Every four days	18.47 p	1.004 p	0.96 p
Every six days	26.33 p	0.809 p	1.38 p
Interaction	(-)	(-)	(-)
CV (%)	14.25	23.62	21.58

Remarks: Based on DMRT ( $\alpha = 0.05$ ), there was no significant difference between the values with the same alphabet in one column, and (-) interaction effects were insignificant.

**Table 3.** Plant dry weight, harvest index, number of filled grains, the weight of 100 grains, and total grains weight per plant under the different weights of rice husk charcoal and interval time of watering

Treatments	Harvest index	Number of filled grains	Weight of 100 grains (g)	Total grains weight (g)
<b>Rice husk charcoal</b>				
0 t ha <sup>-1</sup>	0.11 a	162.28 a	11.83 a	17.12 a
10 t ha <sup>-1</sup>	0.10 a	193.94 a	11.25 a	21.66 a
20 t ha <sup>-1</sup>	0.10 a	217.50 a	10.65 a	25.88 a
<b>Interval time of watering</b>				
Every two days	0.11 p	279.28 p	12.12 p	30.16 p
Every four days	0.10 q	136.83 q	10.83 p	16.14 p
Every six days	0.09 q	157.62 q	10.78 p	18.37 p
Interaction	(-)	(-)	(-)	(-)
CV (%)	18.17	22.25	14.27	23.21

Remarks: Based on DMRT ( $\alpha = 0.05$ ), there was no significant difference between the values with the same alphabet in one column, and (-) interaction effects were insignificant.

Meanwhile, the tolerance mechanism by plants is can regulate the accumulation of osmoprotectants and detoxify ROS by producing antioxidants. Increased ROS along with increased lipid peroxidation disrupts cell membrane function. The plasma membrane is damaged, causing cell loss, shrinking, and then dying (Demidchik, 2015). According to Anjum et al. (2011), lipid peroxidation produces malondialdehyde (MDA) and this is used as a physiological biomarker of drought-stressed in plants.

Proline includes a non-enzymatic antioxidant that helps deradicalization (Ilyas et al., 2021). Research by Ghaffari et al. (2019) also showed consistent results that exogenous proline application in drought-stressed plants was able to prevent lipid membrane peroxidation and reduce the presence of H<sub>2</sub>O<sub>2</sub> better than without proline application. Yaish (2015) also agreed the major role of proline as an electron receptor so that plants could repair the cell damage due to the formation of ROS. From these results, Jali performs an avoidance and tolerance mechanism towards low water availability. The longer the interval of watering, the longer the transpiration time. Prolonged transpiration is the avoidance mechanism to reduce water loss and maintain leaf surface temperature. Then the tolerance mechanism by Jali plants is to produce proline as an enzymatic antioxidant.

Individually, the treatment of RHC had no significant effect on the harvest index, number of filled grains, the weight of 100 grains, and yield per plant (Table 3). With or without the addition of RHC, the decrease in soil moisture content of up to 50% (Table 1) did not give a statistically significant difference to the jali yields. This result indicated that the weight of RHC has not been able to increase the yield of jali under drought stress. Conversely, the treatment of interval time of watering had a significant effect on the harvest index, the number of filled grains, and the weight of the fresh grain per plant but had no significant effect on the weight of 100 filled grains.

The weight of 100 seeds was not significantly different from the decrease in soil moisture content, but the number of filled grains decreased. This supports that when fertilization occurs, the decrease in water availability causes both pollen and pistil to lose their viability. Furthermore, although pollination occurred, the fertilization process failed to form and the number of filled grains decreased. The harvest index also decreased along with the longer interval of watering. The harvest index proves that under

low soil moisture content, the ability of plants to translocate and assimilate from source to sink also decreases. Even so, Jali can defend the generation by producing seeds under the fluctuating minimum soil moisture content (11.85% the lowest at watering every six days). Ruminta et al. (2017) confirmed that drought stress that occurs during the flowering phase of plants reduces pollen viability, thereby reducing the pollination process and the number of grains formed. This will then reduce plant productivity. These results are in line with research by Panda et al. (2017) that drought stress in several rice genotypes caused an increase in leaf relative water content of 31.57% accompanied by an increase in leaf proline content and grain sterility of more than 50%. This caused a decrease in the number of productive tillers so crop yields also decreased by 55.31%. Then, in maize, the length of the watering interval every 3 weeks showed a decrease in the number of cobs, the weight of 100 seeds, and corn yield compared to watering every 2 weeks and once a week (Shinoto et al., 2018).

## CONCLUSIONS

There was no interaction effect between the treatment of rice husk charcoal and the interval time of watering in all observations. The results showed that the addition of rice husk charcoal into the soil did not significantly affect the plant yield and the yield contributing characters under drought stress. Jali plants could still produce grains at an interval time of watering of every six days with a minimum soil moisture content of 11.85%, or equivalent to 35.54% of field capacity.

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