



Effect of Organic Amendments on the Water Stress Resistance of Corn Varieties during Vegetative Stage in Ultisols

B. Bukhari¹, Sabaruddin Zakaria^{1,2}, S. Sufardi^{1,3}, S. Syafruddin^{1,2}

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ABSTRACT

Background: Water stress is one of the most crucial limiting factors in maize production on Ultisols. One solution that could be adopted to solve this problem is applying organic soil amendment.

Methods: This study evaluated three varieties of corn: NK-Jumbo, Srikandi Kuning and Lamuru. The water stress treatment consists of 75% field capacity (without water stress) and 37.5% field capacity (water stress treatment). The organic amendment consists of without soil amendment, rice-straw 10 tons ha⁻¹, biochar 10 tons ha⁻¹ and rice straw 5 tons ha⁻¹ + biochar 5 tons ha⁻¹. A factorial, completely randomized design was used in this research.

Result: Water stress and organic amendments affect total stomata, leaf curl, leaf area, proline content, shoot and root dry weight, shoot-root ratio and biomass production of corn. Applying organic amendments increases corn's resistance to water stress, indicated by decreased plant proline content and increased biomass. The proline content of corn by application of organic amendment with rice straw 5 tons ha⁻¹ + biochar 5 tons ha⁻¹ (70.41 µ mol/g) was lower than the proline content of treatment without amendments (93.69 µ mol/g). NK-Jumbo was the most resistant variety to water stress conditions.

Key words: Corn growth, Organic amendment, Proline content, Water stress.

INTRODUCTION

In Indonesia, corn is generally produced from dryland regions with soils of Ultisols, Inceptisols, Oxisols andisols, Alfisols and Entisols. One of the soil orders targeted for maize development is Ultisols because this soil reaches 25% of the total land area in Indonesia. This soil is present in the tropical zone and characterized by low fertility (Xu *et al.* 2018). Moisture stress is a significant problem in this soil.

Water stress causes plant physiological changes such as reduction in photosynthesis and transpiration, change the physiological characteristics of plant leaves, such as leaf photosynthesis and transpiration rate (Hussain *et al.* 2018) and also cause the reduction of the weight of fresh and dried plants (Huang *et al.* 2010). Planting corn on this soil resulted in a decrease in corn yields if water stress occurred during the generative stage (Mi *et al.* 2018). This problem can be overcome by applying organic amendment in this soil type. The organic amendment significantly affects soil quality improvement, primarily when used with an organic fertilizer (Chuan-Chuan *et al.* 2017).

Applying organic amendment can promote decomposition in the mineralization process in Ultisol and increase the activity of soil micro-organisms (Xu *et al.*, 2020; Elayaraja and Sathiyamurthi, 2020). Biochar, manure as well charcoal and compost are organic amendments that can improve soil quality and crop production (Hussain *et al.*, 2017; Triatmoko *et al.*, 2020; Nasar *et al.*, 2019), which plays a role in absorbing and neutralizing harmful contaminants in the soil (Yuan *et al.* 2019). All types of biochar can improve groundwater availability, including clay (Günel *et al.* 2018). At present, a lack of information has been found regarding the use of organic amendments to the resistance of corn plants to water stress, especially on ultisols. Here, this study

¹Doctoral Program in Agricultural Science, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia.

²Department of Agrotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia.

³Department of Soil Science, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia.

Corresponding Authors: B. Bukhari and Sabaruddin Zakaria, Doctoral Program in Agricultural Science, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia.

Email: bukharimp@gmail.com; zaksabar@unsyiah.ac.id.

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was carried out to examine the effect of organic amendment on physiological parameters and biomass of corn under water stress. The study evaluated these treatments' single and interactive effect on corn physiological parameters and biomass production during the vegetative stage. This study has tested three main hypotheses: 1) Among the varieties showed different physiological parameters and biomass production, 2) Water stress affects physiological and biomass production of corn and 3) Organic amendment could increase the resistance of water stress in Ultisols.

MATERIALS AND METHODS

Site location, source of Ultisol and genetic materials

The ultisols soil was taken from Aceh Besar District, Janthoe sub-district with 0.5°17'5.2"N and 95°35'12"E coordinates.

The research was carried out in the greenhouse of the Agriculture Faculty, Syiah Kuala University, Banda Aceh, Indonesia, from June to December 2019. The seeds of three corn varieties consisting of NK-Jumbo, Srikandi Kuning and Lamuru obtained from Maros Corn Research Institute, South Sulawesi, were used in this research.

Water stress treatment procedure and organic amendment

Water stress treatment refers to the procedure (Xue *et al.* 2012) by weighing and recording the weight of the pot containing air dry ultisol soil before planting the corn seed. The pots were filled with water until saturated and then the pots were weighed, re-recorded and drained for 48 hours. Weight gain was the weight of water used as a benchmark for field capacity conditions; the amount of water that increased was 1.96 liters. So that the water needed for control treatment was $0.75 \times 1.96 = 1.47$ liters and the amount of water needed for water stress treatment was $0.375 \times 1.96 = 0.73$ liters. Organic amendments used in this research were rice straw and biochar.

Experimental design

The research was conducted using a $3 \times 2 \times 4$ factorial design with varieties as first factors consisting of NK-Jumbo, Srikandi Kuning and Lamuru. Water stress is the second factor without water stress (75% of field capacity) and water stress with 37.5% of field capacity. The third factor to be analyzed was the organic amendment consisting of without soil amendment, rice-straw 10 tons ha^{-1} , biochar 10 tons ha^{-1} , rice straw 5 tons ha^{-1} +biochar 5 tons ha^{-1} . The total combination of treatments was 24 with three replications, so there were 72 pots to implement this research. The pot with 30 cm in diameter and height of 40 cm was then applied organic amendments based on the treatments. Each pot was filled only with 15 kg wind dry ultisol without organic amendment treatment. For the treatment of 10 tons of rice straw and 10 tons of biochar ha^{-1} , each pot was filled with wind dry ultisol 14,925 kg with 0.075 kg rice straw and 0.075 kg biochar. Whereas for the treatment with 5 tons of rice straw+5 tons of biochar ha^{-1} , each pot was filled with ultisol 14,925+0.0375 kg rice straw+0.0375 kg biochar.

Each pot was planted with five seeds with a distance of 7.5 cm spacing and only two corns seedlings were maintained to grow in each pot at eight days after planting. Fertilizer was applied with 20 g NPK per pot at planting time and pests were controlled mechanically. On the tenth day after planting, the water stress treatment was carried out and soil moisture was controlled with an electronic balance every two days. The study lasted 40 days after planting.

Agro-physiological character analysis

The analysis for agro-physiological characters consisted of total stomata, leaf area, leaf curls and proline content. Total stomata were measured from the base, middle and tip sections on leaves of maize. Three slides were prepared for each taxon. The total of stomata (mm^2) was calculated on ten representative fields of leaves according to the

method described by (Orsini *et al.*, 2012). Leaf width was measured by foto-electric (leaf area meter LI-3000A). The number of leaf curls was observed manually at 35 days after planting at 12:00-14:00 a clock.

In contrast, proline content was observed in leaves according to the method described by (Bates *et al.* 1973). Proline of approximately 0.5 g of fresh leaves was extracted with 10 ml 3% sulfosalicylic acid and then it was filtrated using Whatman paper. About 2 ml of filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction was abolished in an ice bath. Samples with known proline amounts were assayed in parallel to obtain a standard curve. The absorbance of the supernatants was read at 520 nm using toluene as a blank. Proline concentration was finally expressed as $\mu\text{mol g}^{-1}$ dry weight.

Biomass production analysis.

Biomass production variables consist of shoot dry weight, root dry weight, shoot-root ratio and total biomass. The shoot dry weight is the part corn plant above the soil surface from the root neck; the root dry weight is the bottom of the corn plant in carefully disassembled pots. The shoot and root dried at a temperature of 70°C until the weight was constant and the weight determined the shoot-root ratio in each treatment. In contrast, total biomass is total shoots and roots dry weight, which are weighed after being dried at a temperature of 70°C .

Procedure for data analysis

Data were analyzed by three-way ANOVA using SPSS 21.0 (SPSS Inc) and the significant difference was followed by the LSD (Least significant difference) test.

RESULTS AND DISCUSSION

Agro-physiological character analysis

The effect of water stress and organic amendment on the agro-physiological character of corn varieties is shown in Table 1. The table showed significant differences in total stomata, leaf area and proline content among the varieties of corn. NK Jumbo showed the highest total stomata, leaf area and proline content, whereas Lamuru had the lowest total stomata, leaf area and proline content. The total stomata of Srikandi Kuning and Lamuru were 4.13 and 4.36%, respectively, more diminutive than the total stomata NK-Jumbo.

The table also showed that water stress caused a significant increase in the number of leaf curl and a decrease in leaf area compared to control. The leaf curl and leaf area by water stress treatment were 2.67 and $398.13 \text{ cm}^2/\text{stem}$, respectively. Without water stress treatment, leaf curl and leaf area were 2.44 and $398.13 \text{ cm}^2/\text{stem}$, respectively.

Table 1 also showed that organic amendment caused significant differences in total stomata, leaf curl, leaf area and proline content. Application of rice straw 5 tons ha^{-1} + biochar 5 tons ha^{-1} as soil amendment caused the increase

in total stomata and leaf area to 10.51% and 7.70%, respectively, compared without soil amendment. Application of rice straw 5 ton ha⁻¹+biochar 5 ton ha⁻¹ as soil amendment also caused the decrease in the number of leaf curl and proline content to 39.38% and 33.06%, respectively. In comparison, proline content was significantly increased by water stress condition to be 85.15 µmol.g⁻¹ plant tissue compared to control with only 78.72 µmol.g⁻¹ plant tissue. Proline is one of the secondary metabolites produced by corn under stress (Fukami *et al.* 2018). Drought stress increased proline content and carbohydrate solution in the leaves (Al-Yasi *et al.* 2020).

The proline content in the varieties of NK-Jumbo was higher compared to Lamuru. By applying organic amendment, the proline content decreases substantially compared to without soil amendment. On the other hand, the proline content decreased when organic amendment soil was added to the soil. It is thought that soil amendment with rice straw 5 ton ha⁻¹ + Biochar 5 ton ha⁻¹ was able to avoid water shortages.

Biomass production

The effect of water stress and organic amendment on the biomass production of corn is shown in Table 2. The table shows that the varieties of corn did not show significant differences in biomass production. The table also shows that water stress treatment caused a significant effect on root dry weight and shoot-root ratio. Water stress caused the increasing root dry weight and shoot root ratio to 5.22% and 5.94%, respectively.

Table 2 also showed that organic amendment had a significant effect on all biomass production variables. Application of organic amendment with rice straw 5 tons ha⁻¹ +Biochar 5 tons ha⁻¹ caused significantly higher shoot dry weight, shoot root ratio and total biomass than other amendment treatments. Shoot dry weight, root dry weight, shoot root ratio and total biomass increase for about 33.88%, 18.32%, 12.09%, 28.40%, respectively, compared without organic amendment.

The decreasing of water stress impact and increasing biomass production by organic amendments is thought to be related to improving soil properties by this treatment. Biochar application significantly ameliorated soil physical properties such as bulk density, total porosity, water retention and penetration resistance. Moreover, biochar or organic material could increase soil water availability (Saffari *et al.*, 2021) (Sachan *et al.*, 2020). Applying biochar as soil amendment enhanced carbon assimilation in soybean, resulting in increased biomass accumulation and yield (Zhu *et al.* 2019).

Effect interaction between a variety of corn and water stress treatment

Drought affects more or less at every growth stage, causing a reduction of plant growth, yield components and yield (Moonmoon and Islam, 2017). Effect the interaction between variety and water stress treatment is shown in Table 3. The

table showed that water stress caused a decrease in leaf area for about 1.2-6.5% and increased proline content for about 3.9-10.9% in all the varieties. Moreover, water stress also caused a significant decrease in dry shoot weight in Lamuru from 165, 8 to 154,95 g. stem⁻¹. In NK Jumbo and Srikandi Kuning, water stress did not significantly impact the shoot dry weight and total biomass.

Effect interaction between varieties of corn and organic amendment

Effect the interaction between varieties and the organic amendment is shown in Table 4. The table showed that organic amendment with rice straw 5 tons ha⁻¹+Biochar 5 tons ha⁻¹ caused a significant increase in leaf area, proline content, shoot dry weight, root dry weight, shoot root ratio and total biomass compared to other organic amendments. Leaf area, shoot dry weight, root dry weight, shoot-root ratio and total biomass increase for about 3.49-11.58%, 25.12-25.18%, 12.24-18.34%, 8.16-12.81% and 20.93-23.98%, respectively compare without organic amendment. Moreover, organic amendment with rice straw 5 tons ha⁻¹ + Biochar 5 tons ha⁻¹ caused decreasing of proline content in NK Jumbo (31.23%), Srikandi Kuning (18.62%) and Laura (24.10%) compared to without soil amendment.

Biochar as soil amendments significantly improved photosynthetic characteristics in maize (Wang *et al.* 2021). The increasing of leaf area, shoot dry weight, root dry weight, shoot-root ratio and total biomass and the decreasing of proline content by organic amendment with rice straw 5 tons ha⁻¹+Biochar 5 tons ha⁻¹ in all varieties of corn indicate the superiority of this organic amendment. In contrast, the decreasing proline accumulation by organic amendment is caused by lower activities of-pyrroline-5-carboxylate synthetase. The study suggests that proline accumulation in maize leaves under water stress can be explained by the higher enhanced activities of-pyrroline-5-carboxylate synthetase and higher inhibition of proline dehydrogenase (Zhang *et al.* 2017).

Effect interaction between water stress and organic amendment treatment

Effect the interaction between water stress and organic treatment is shown in Table 5. The table showed that organic amendment caused the increase in leaf area, shoot dry weight, shoot-root ratio and total biomass. However, this treatment caused a reduction in proline content. The decrease in proline content occurred both under stress and non-stress treatment. Organic amendment with rice-straw 5 tons ha⁻¹+Biochar 5 tons ha⁻¹ caused the increase in leaf area, shoot dry weight, shoot root ratio and total biomass for about 9.39%, 38.76%, 17.87%, 32.55%, respectively, under control condition. Whereas in water stress conditions, organic amendment with rice-straw 5 tons ha⁻¹+Biochar 5 tons ha⁻¹ caused the increase in leaf area, shoot dry weight, shoot root ratio and total biomass for about 5.98%, 30.21%, 6.25%, 24.26%, respectively compared to without organic amendment. Moreover, organic amendment with rice-straw

Table 1: Effect of water stress and organic amendment on agro-physiological characters of three maize varieties at vegetative stage.

	Total stomata (no/mm ²)	Number of leaf curls (strands/stems)	Leaf area (cm ² /stems)	Prolin content (µmol/g plant tissue)	F table	
					0.01	0.05
Varieties						
NK-Jumbo	96.75b	2.63a	405.69b	86.02b	3.18	5.08
Srikandi Kuning	92.92a	2.60a	419.13c	82.81ab	3.18	5.08
Laura	92.71a	2.44a	387.57a	76.98a	3.18	5.08
Water stress						
Control (75% FC)	94.54a	2.44a	410.13b	78.72a	4.03	7.17
Water stress (37.5% FC)	93.67a	2.67b	398.13a	85.15b	4.03	7.17
Organic amendments						
No amendment	92.00ab	3.25c	394.82a	93.69b	2.79	4.20
Rice-straw	93.28b	2.67bc	401.91ab	80.23a	2.79	4.20
Biochar	89.56a	2.33b	394.58a	83.67b	2.79	4.20
Rice-straw+Biochar	101.67c	1.97a	425.22b	70.41a	2.79	4.20
Anova						
V	6.52**	2.00ns	19.58**	17.74**	3.18	5.08
S	0.79ns	4.91*	6.00*	26.14**	4.03	7.17
B	26.13**	30.44**	6.99**	59.77**	2.79	4.20
V×S	0.60ns	1.90ns	5.98**	1.20ns	3.18	5.08
V×B	1.02ns	0.44ns	3.03*	3.64**	2.29	3.18
S×B	2.50ns	0.96ns	3.69*	4.69*	2.79	4.20
V×S×B	1.83ns	0.42ns	4.16**	2.18ns	2.29	3.18

V= Variety, S= Water stress, B= Organic amandemen, V×S= Variety and water stress interaction, V×B= Variety and organic mandemen interaction, S×B= Water stress and organic amandemen interaction, V×S×B= Variety × water stress × organic amandemen interaction; µmol/g= Micro mol per gram; ns= Non significant different, *= Significant different, **= Highly significant different; a,b,c= Difference effect LSD test level at P=0.05.

Table 2: Effect of water stress and organic amendment on biomass production characters of three maize varieties at the vegetative stage.

	Shoot dry weight (g)	Root dry weight (g)	Shoot-root ratio	Total Biomassa (g)	F tabel	
					0.05	0.01
Varieties						
NK-Jumbo	163.77a	72.49a	2.25a	237.00a	3.18	5.08
Srikandi Kuning	160.97a	71.76a	2.26a	233.43a	3.18	5.08
Laura	160.45a	71.55a	2.25a	232.40a	3.18	5.08
Drought stress						
Control (75% FC)	161.51a	70.10a	2.19a	236.50a	4.03	7.17
Water stress (37.5% FC)	161.95a	73.76b	2.32b	232.04a	4.03	7.17
Organic amendment						
No amendement	135.42a	63.64a	2.15a	200.02a	2.79	4.20
Rice-straw	166.85b	75.59b	2.21ab	233.43bc	2.79	4.20
Biochar	163.35b	73.13b	2.24b	236.81b	2.79	4.20
Rice-straw + biochar	181.30c	75.37b	2.41c	256.83c	2.79	4.20
Anova						
V	1.50 ns	1.31ns	0.07ns	1.48ns	3.18	5.08
S	0.07 ns	54.94**	19.60**	3.78ns	4.03	7.17
B	129.49**	129.98**	13.71**	120.89**	2.79	4.20
V×S	8.87**	2.09ns	2.31ns	6.98**	3.18	5.08
V×B	4.05**	3.28**	3.11*	4.11**	2.29	3.18
S×B	10.96**	1.06ns	8.11**	3.53*	2.79	4.20
V×S×B	3.64**	1.01ns	2.88*	2.54*	2.29	3.18

V= Variety, S= Stres air, B= Organic amandemen, V×S= Variety and water stress interaction, V×B= Variety and organic mandemen interaction, S×B= Water stress and organic amandemen interaction, V×S×B= Variety × water stress × organic amandemen interaction; ns= Non significant different, *= Significant different, **= Highly significant different; a,b,c= Difference effect LSD 0.05.

Table 3: Effect of water stress on agro-physiological characters of three corn varieties at vegetative stage.

Water stress treatment	Leaf area (cm ² /stems)	Proline content (µmol/g plant tissue)	Shoot dry weight (g/stems)	Total biomass (g)
NK-Jumbo				
Control (75% FC)	408.10cd	81.83b	162.35ab	232.43ab
Water stress (37.5% FC)	403.28cd	90.20c	168.83b	241.56b
Srikandi Kuning				
Control (75% FC)	422.51e	80.93b	167.58b	227.53a
Water stress (37.5% FC)	415.75de	84.09bc	164.44b	239.33ab
Laura				
Control (75% FC)	399.78b	73.40a	165.82b	236.18ab
Water stress (37.5% FC)	375.37a	80.55b	154.95a	228.62a
LSD 0.05	12.08	6.19	8.46	11.29

Numbers followed by the same letter in the same column for each corn variety were not significantly different according to the LSD test of 0.05; FC= Field capacity.

Table 4: Effect of organic amendment on agro-physiological and biomass character of three corn varieties at vegetative stage.

Organic amendment	Leaf area (cm ² /stems)	Proline content (µmol/g plant tissue)	Shoot dry weight (g/stems)	Root dry weight (g/stems)	Shoot-root ratio	Total Biomass (g)
NK-Jumbo						
No amendment	392.78b	101.67e	139.50b	63.67ab	2.25ab	199.46a
Rice-straw (10 t ha ⁻¹)	406.50d	81.33c	161.84cd	76.55e	2.21ab	247.12e
Biochar (10 t ha ⁻¹)	395.46bc	91.83d	156.25c	73.14cd	2.10a	246.36e
Rice-straw 5 t ha ⁻¹ + Biochar 5 t ha ⁻¹	444.26f	69.23a	186.30f	76.76e	2.45b	262.39g
Srikandi Kuning						
No amendment	402.31cd	91.31b	133.00a	65.19b	2.11a	198.95a
Rice-straw (10 t ha ⁻¹)	407.70d	82.83c	171.44cd	76.09e	2.25ab	247.89e
Biochar (10 t ha ⁻¹)	422.26de	82.77b	171.80cd	71.82c	2.34b	233.82d
Rice-straw 5 t ha ⁻¹ + Biochar 5 t ha ⁻¹	428.03e	74.31a	178.84ef	74.28de	2.36ab	253.06de
Laura						
No amendment	389.37b	88.10d	133.76ab	62.06a	2.11a	201.66ab
Rice-straw (10 t ha ⁻¹)	391.52b	76.53b	167.27d	73.44cd	2.19ab	235.28 d
Biochar (10 t ha ⁻¹)	366.03a	76.40b	182.00ef	74.02cd	2.29ab	230.27c
Rice-straw 5 t ha ⁻¹ + Biochar 5 t ha ⁻¹	403.38cd	66.87a	178.78ef	75.72de	2.42b	255.05f
LSD 0.05	12.08	6.19	8.30	2.44	0.15	1.29

Numbers followed by the same letter in the same column for each corn variety were not significantly different according to the LSD test of 0.05.

Table 5: Effect interaction between water stress and organic amendment on agro-physiological and biomass production in three varieties of corn at the vegetative stage.

Amendment organic application	Leaf area (cm ² /stems)	Prolin content (µmol/g plant tissu)	Shoot dry weight(g/stems)	Shoot-root ratio	Total Biomass (g)
Control (75% FC)					
No amendment	397.84b	88.11cd	135.16a	2.07a	199.77a
Rice-straw (10 t ha ⁻¹)	401.62bc	74.92ab	159.48b	2.04a	242.49bc
Biochar (10 t ha ⁻¹)	405.85c	83.74c	163.87c	2.20b	238.96b
Rice-straw 5 t ha ⁻¹ +Biochar 5 t ha ⁻¹	435.21e	68.11a	187.55e	2.44b	264.80d
Water stress (37.5% FC)					
No amendment	391.80ab	99.28e	134.44a	2.24b	200.27a
Rice-straw (10 t ha ⁻¹)	402.19c	85.54cd	174.22d	2.39b	244.38bc
Biochar (10 t ha ⁻¹)	383.31a	83.59cd	162.84bc	2.28ab	234.66bc
Rice-straw 5 t ha ⁻¹ +Biochar 5 t ha ⁻¹	415.23d	72.18ab	175.06d	2.38 b	248.86c
LSD 0.05	9.86	6.19	6.91	0.12	9.22

Numbers followed by the same letter in the same column for each corn variety were not significantly different according to the LSD test of 0.05; FC= Field capacity.

5 tons ha⁻¹+ Biochar 5 tons ha⁻¹ caused the decrease in proline content to 27.30% and 22.70% in water stress and non-stress condition, respectively, compared without organic amendment. The organic amendment can reduce stress; this is evident through the decrease in proline content and an increase of all variables compared to other organic amendments in the three varieties of corn with decreasing proline content.

CONCLUSION

The varieties of corn showed different adaptations to water stress conditions. Water stress significantly affects the agro-physiological character and biomass of this plant. Moreover, application of rice-straw 5 tons ha⁻¹+Biochar 5 tons ha⁻¹ as organic amendment significantly increased in total stomata, leaf area, shoot and root dry weight, shoot-root ratio and total biomass and caused the decrease in proline content to 70.41 µmol.g⁻¹ plant tissue compared without organic amendment (93.69 µmol.g⁻¹ plant tissue). NK-Jumbo was the most tolerant variety to water stress conditions and had a proline content of 90.2 µmol/g plant tissue by water stress condition.

Conflict of interest: None.

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